The companion of cellulose synthase 1 confers salt tolerance through a Tau-like mechanism in plants

Christopher Kesten1,2,3, Arndt Wallmann4, René Schneider2,3, Heather E. McFarlane2, Anne Diehl4, Ghazanfar Abbas Khan2, Barth-Jan van Rossum4, Edwin R. Lampugnani2, Witold G. Szymanski3, Nils Cremer4, Peter Schmieder4, Kristina L. Ford2, Florian Seiter4, Joshua L. Heazlewood2, Clara Sanchez-Rodriguez1, Hartmut Oschkinat4 & Staffan Persson2,3

Microtubules are filamentous structures necessary for cell division, motility and morphology, with dynamics critically regulated by microtubule-associated proteins (MAPs). Here we outline the molecular mechanism by which the MAP, COMPANION OF CELLULOSE SYNTHASE1 (CC1), controls microtubule bundling and dynamics to sustain plant growth under salt stress. CC1 contains an intrinsically disordered N-terminus that links microtubules at evenly distributed points through four conserved hydrophobic regions. By NMR and live cell analyses we reveal that two neighboring residues in the first hydrophobic binding motif are crucial for the microtubule interaction. The microtubule-binding mechanism of CC1 is reminiscent to that of the prominent neuropathology-related protein Tau, indicating evolutionary convergence of MAP functions across animal and plant cells.
Microtubules are tubular structures essential to morphogenesis, division and motility in eukaryotic cells. While animal cells typically contain a centrosome with radiating microtubules toward the cell periphery, growing plant cells arrange their microtubules along the cell cortex. A major function of the cortical microtubules in plant cells is to direct the synthesis of cellulose, a fundamental component of the cell wall essential to plant morphology. Cellulose is produced at the plasma membrane by cellulose synthase (CESA) protein complexes (CSCs) that display catalytically driven motility along the membrane. The recently described microtubule-associated protein (MAP), COMPANION OF CELLULOSE SYNTHASE 1 (CC1), is an integral component of the CSC and sustains cellulose synthesis by promoting the formation of a stress-tolerant microtubule array during salt stress. As cellulose synthesis is key for plant growth, engineering of plants to better produce cellulose is of utmost importance to agriculture. Indeed, understanding the molecular mechanism by which CC1 controls cellulose synthesis may bear opportunities to improve cultivation on salt-affected lands.

The microtubule network is highly dynamic, and its state is influenced by the action of MAPs. The mammalian Tau/MAP2/MAP4 family represents the most investigated MAP set, primarily due to Tau’s importance in the pathology of neurodegenerative diseases. In vitro, Tau promotes polymerization and bundling of microtubules, and diffuses along the microtubule lattice. In the brain, Tau is predominantly located at the axons of neurons, where it contributes to the microtubule organization that drives neurite outgrowth. In disease, Tau self-aggregates into neurofibrillary tangles that might trigger neurodegeneration. Intriguingly, no clear homologs of the Tau/MAP2/MAP4 family have been identified in plants. Because, the full scope of Tau’s biological role remains elusive, identification of Tau-related proteins outside the animal Kingdom would benefit our understanding of how this class of MAPs functions.

In this study, we unravel the microtubule-binding mechanism of CC1 and show that it is reminiscent to that of Tau, indicating evolutionary convergence of MAP functions across animal and plant cells.

**Results**

The N-terminus of CC1 bundles microtubules. The cytosolic N-terminal part of CC1 (residues 1–120, CC1ΔC223) binds to microtubules and restores microtubule reassembly, cellulose synthesis and wild-type growth of cclce2 (null-mutation in CC1 and its closest homolog CC2) seedlings on high levels of salt. These data indicate that CC1ΔC223 is critical to CC1’s function during stress, and we therefore set out to investigate the molecular details of how it interacts with microtubules. We cross-linked 6xHis-tagged CC1ΔC223 with α-β-tubulin dimers using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), which led to di- and multimeric protein products (Fig. 1a, Supplementary Fig. 1a). We used EDC, which links functional groups of lysine to either aspartate or glutamate, and not the typical sulfhydryl-reactive or lysine–lysine cross-linkers, as CC1ΔC223 only contains a single cysteine and has a basic isoelectric point (pI) (i.e. the main reactive amino acid is lysine), while tubulin/microtubules have an acidic pI (i.e. the main reactive amino acids on the surface of the tubulin dimer are aspartate and glutamate). After LC/MS/MS analysis, we used four different software packages (StavroX, pLink, SIM-XL, Crux) to identify potential inter-cross-links between tubulin and CC1ΔC223. Extensive manual curation resulted in five well-defined covalent bonds between CC1ΔC223 and α- or β-tubulin (Fig. 1b). We consistently detected four peptides of CC1ΔC223 cross-linked to β-tubulin (K40–E111, K94–E111, K96–E111 and K96–E158; letters and numbers indicate amino acids in CC1ΔC223 and β-tubulin, respectively; Fig. 1b, c; Supplementary Table 2 and Supplementary Fig. 1b-h). Notably, the three sequentially distant K40 and K94/96 of CC1ΔC223 cross-linked to the same residue on β-tubulin (E111). This suggests that two CC1 regions might bind the same sites on two different β-tubulin molecules, which is corroborated by the multimeric protein products in the SDS page. The cross-linked position on α-tubulin is close to the hydrophobic interface between tubulin heterodimers, a site that is frequently occupied by agents that directly regulate microtubule formation, such as vinblastine, the stathmin-like domain (SLD) of RB3, and also by Tau.

To further investigate how CC1ΔC223 binds microtubules, we co-polymerized tubulin in the presence of CC1ΔC223. We then labeled CC1ΔC223 using 5 nm gold-conjugates that recognize the His-tag and monitored the formed microtubules and gold distribution via transmission electron microscopy (TEM). Gold labeling only occurred at closely aligned microtubules with very small inter-microtubule distances (Fig. 1d, e, Supplementary Fig. 1d-e), and were visible as evenly distributed foci in straight rows along interphases of two neighboring microtubules (Fig. 1d, e), while a negative control employing BSA did not show any specific microtubule labeling (Supplementary Fig. 2). The gold particles were typically spaced by 10 nm (Fig. 1f; 10.0 nm ± 2.4 nm; mean ± S.D.; three-independent experiments; n = 1785 labels). The number of gold-labels in a given row ranged between two and 41 labels (Fig. 1g; 8 ± 5 labels; mean ± S.D.; three-independent replicates; n = 274 rows), making each row about 80 nm in length. We also observed multiple gold-labeled rows on one microtubule when in close proximity to several other microtubules (Supplementary Fig. 2d). The angles between gold-labeled rows were small (Fig. 1h; 2.8° ± 3°; mean ± S.D.; three-independent replicates; n = 98 rows), highlighting that the labeling did not shift between neighboring protofilaments on the same microtubule. These data indicate that CC1ΔC223 promotes microtubule bundling. Indeed, increasing levels of CC1ΔC223 correlated with increased microtubule bundling in TEM experiments (Fig. 2a, b), while a BSA control did not show increased bundling (Supplementary Fig. 3a, b).

The CC1 N-terminus can diffuse along the microtubule lattice. As our TEM experiments only provide static information on the interactions between CC1ΔC223 and microtubules, we labelled the sole sulphhydryl group (C119) in CC1ΔC223 with the green fluorescent dye CF488A-maleimide (Supplementary Fig. 3c) and performed rhodamine-labeled microtubule interaction assays. Using total internal reflection fluorescence microscopy, we observed most of the CF488A-labeled CC1ΔC223 proteins as fluorescent foci associated with microtubules (Fig. 2c, Supplementary Movie 1). CF488A-labeled CC1ΔC223 diffused bidirectionally along the rhodamine-labeled microtubules and occurred on both single and bundled microtubules (Fig. 2d). In accordance with the results above, CF488A-labeled foci occupied bundled microtubules for a mean ± S.D. of 0.007 ± 0.007 μm² s⁻¹; mean ± S.D.; n = 50 molecules) of fluorescent foci exhibited a linear relationship with time (Supplementary Fig. 3c), indicating free diffusion. These data are reminiscent to that of Tau, which promotes microtubule-bundling and polymerization, and also moves along microtubules in vitro with comparable diffusion coefficients (0.142–0.292 μm² s⁻¹).

The N-terminus of CC1 is intrinsically unstructured. To understand how CC1ΔC223 engages with microtubules, we
Fig. 1 The N-terminus of CC1 binds sites on both α- and β-Tubulin and cross-links microtubules. a SDS-Page of EDC-induced cross-linking of 6xHis-CC1ΔC223 (16 kDa) and tubulin dimers (2 × 55 kDa). Arrowheads depict position of relevant protein bands. MW molecular weight marker, CL1-4 cross-linking reaction 1–4. Higher order cross-linking products represent cross-links between e.g. tubulin +2 × CC1ΔC223 (87 kDa), tubulin dimers (110 kDa), tubulin dimers + CC1ΔC223 (126 kDa), tubulin dimers +2 × CC1ΔC223 (142 kDa). b Schematic views of the secondary structures of α- and β-tubulin, and the CC1ΔC223 sequence. Dashed lines depict detected cross-linking positions of CC1ΔC223 and α- or β-tubulin. c Projection of detected cross-links onto an α/β-tubulin dimer (PDB code 1tub). Dark blue = α-tubulin; Light blue = β-tubulin; Sites for cross-linked amino acids are marked in red. d Representative TEM image of CC1ΔC223 distribution along negatively stained, taxol-stabilized microtubules polymerized in the presence of 6xHis-CC1ΔC223. CC1ΔC223 protein is visualized by a 5 nm gold-conjugated Ni-NTA tag that recognizes 6xHis-tagged proteins. A transect was taken along rows of gold particles, and dips in the light intensity along the transect correspond to gold particle centers. Note the even distribution of the electron-dense gold particles in between neighboring microtubules. Scale bar = 50 nm. e CC1ΔC223 distribution along negatively stained, taxol-stabilized microtubules polymerized in the presence of 6xHis-CC1ΔC223. CC1ΔC223 can form a zipper-like pattern that links microtubules. Scale bar = 100 nm. f Quantification of the distance between individual gold particles as shown in d and e (box plot: Center lines show the medians; box limits indicate the 25th and 75th percentiles; whiskers extend to the minimum and maximum). g, h Quantification of number of gold labels per row (g) and the angle between adjacent gold-labeled rows (h) from images as those in d and e (box plots: Center lines show the medians; box limits indicate the 25th and 75th percentiles; whiskers extend to the minimum and maximum).
assessed its structural features using "solution state" NMR, circular dichroism spectroscopy (CD) and analytical ultracentrifugation (AUC). The 2D 1H–15N-heteronuclear single quantum coherence (HSQC) spectrum of 15N-labeled CC1ΔC223 showed narrow signals and poor chemical shift dispersion in the 1H dimension, which is characteristic for intrinsically disordered proteins (Fig. 3a). For the sequence-specific assignment, we used a combination of three-dimensional and four-dimensional experiments with non-uniform sampling to assign ~85% of the backbone resonances. The disordered nature of CC1ΔC223 in the presence and absence of taxol-stabilized microtubules (magenta) in vitro. Scale bar = 5 μm. b Time-series images (left panel) of CF488A-labeled 6xHis-CC1ΔC223 (green) diffusing along microtubules (magenta). Filled arrow = position in current frame, empty arrow = position in previous frame. Scale bar = 2 μm. c CF488A-labeled 6xHis-CC1ΔC223 proteins (green) associated with surface-bound microtubules (magenta) in vitro. Scale bar = 5 μm. d Time-series images (left panel) of CF488A-labeled 6xHis-CC1ΔC223 (green) diffusing along microtubules (magenta). Filled arrow = position in current frame, empty arrow = position in previous frame. Scale bar = 2 μm. Representative kymograph (right panel) along solid line in left panel (top) showing diffusion of 6xHis-CC1ΔC223 foci. Scale bar = 2 μm. e 6xHis-CC1ΔC223 lifetime on single versus bundled microtubules (box plots: Center lines show the medians; box limits indicate the 25th and 75th percentiles; whiskers extend to the minimum and maximum), n = 60 single and 37 bundled microtubules, ***p-value < 0.001, Welch’s unpaired t-test.

**Fig. 2** The N-terminus of CC1 induces microtubule bundling and can diffuse along the microtubule lattice. a Transmission electron microscopy (TEM) of negatively stained taxol-stabilized microtubules after addition of increasing levels of 6xHis-CC1ΔC223 during microtubule polymerization. Note that it is very difficult to discern individual microtubules in the microtubule bundles after addition of ~3 μM of CC1ΔC223. Scale bars = 100 nm. b Quantification of the proportion of microtubules in bundles (left y-axis, box plots: Center lines show the medians; box limits indicate the 25th and 75th percentiles; whiskers extend to the 10th and 90th percentiles, outliers are represented by dots) and number of microtubules/bundle (right y-axis, magenta line: mean ± SEM) with increasing concentration of 6xHis-CC1ΔC223 (quantified from images such as those in a). c Representative kymograph (right panel) along solid line in left panel (top) showing diffusion of 6xHis-CC1ΔC223 foci. Scale bar = 2 μm. Single MTs; Bundled MTs.

**CC1 engages with microtubules via four hydrophobic motifs.** To study CC1ΔC223-microtubule interactions in a residue-specific manner, we recorded 1H–15N HSQC spectra of 15N-labeled CC1ΔC223 in the presence and absence of taxol-stabilized microtubules. We observed line broadening and vanishing of individual cross-peaks when microtubules were added (Fig. 4a, b). The effects of the microtubules on the transverse relaxation rate (ΔR₂) of CC1ΔC223 signals were reversible, residue specific, independent of the magnetic field, did not correlate with the chemical shift changes, and relaxation dispersion experiments did not show contributions of intermediate exchange (Supplementary Fig. 5a-g). To conclude, the line broadening is a direct result of CC1ΔC223-microtubule complex formation. Figure 4c shows the intensity ratios of cross-peaks taken from 3D HNCA spectra of 15N,13C-labeled CC1ΔC223 in the presence and the absence of 6xHis-CC1ΔC223 bound/microtubules (b/b₀) per residue. A significant intensity decrease is observed in four regions, comprising residues 22RPVYYVQ50, 43FHSTPV1SPM54, 74FSGSLKPG83 and 103QWKECAVI110 (Fig. 4c). Due to signal overlap, the region between residues 60 and 80 is not well covered. We found a clear correlation between the NMR-based microtubule-interaction profile and the hydrophobicity pattern of CC1ΔC223, highlighting the role of hydrophobic interactions (Fig. 4d). The binding motifs are separated by stretches of mobile residues, presumably acting as linkers that are likely to retain a high degree of
Two tyrosine residues contribute to CC1 microtubule binding.

Microtubule binding of the four regions individually was investigated by saturation transfer difference (STD) NMR measurements (Supplementary Fig. 6a). The peptides CC1(16–38), CC1(41–64), CC1(65–85) and a positive control peptide Tau (211–242) yielded strong STD intensities in the amide and aromatic regions of the 1H spectrum (Supplementary Fig. 6b–e). No significant STD effects were observed for a negative control peptide, CC1(83–100), corresponding to the third poorly conserved linker region, and for the most C-terminal region CC1(100–114) (Supplementary Fig. 6f–g). Targeting the N-terminal binding site, the exchange of 3TYY7 to alanine in a CC1YYAA (16–38) peptide resulted in a substantially reduced STD profile, corroborating a contribution of these aromatic rings to the interaction (Supplementary Fig. 6h). Indeed, the same mutation in CC1ΔC223 resulted in significantly reduced signal broadening of residues in the N-terminal region, while the intensity ratios for the C-terminal part remained similar to the wild-type protein (Fig. 4f). Likewise, the mutated CC1ΔC223 bound to microtubules with a lower affinity compared to the wild-type sequence in microtubule spin down assays (Supplementary Fig. 6i–j), corroborating an important function of the two tyrosine residues in microtubule binding.

Mutation of CC1 impairs CESA movement. To assess how mutations in the two microtubule-binding tyrosine residues affect the function of CC1 in vivo, we mutated them to alanine in the full-length CC1 (CC1YYAA), fused it N-terminally with GFP, and transformed it into Arabidopsis thaliana cclec2 mutant plants. The cclec2 mutant seedlings display reduced growth and crystalline cellulose content on salt-containing media5. These phenotypes were not restored in cclec2 GFP-CC1YYAA seedlings when grown on salt-containing media as compared to controls (Fig. 5a–c).

Spinning-disc confocal microscopy showed GFP-CC1YYAA signals as distinct foci at the plasma membrane (Supplementary Movie 2) and within cytoplasmic compartments in dark-grown Arabidopsis hypocotyl cells, in accordance with reports on GFP-CC15 (Supplementary Fig. 7a–c). GFP-CC1 co-localizes and migrates with tdTomato(tdT)-CESA6, which is an important subunit of the CSC30, at the plasma membrane5. Notably, the GFP-CC1YYAA also co-migrated with tdt-CESA6 at the plasma membrane on MS media without addition of salt (Supplementary Fig. 7a–b, d–e; Pearson correlation coefficient $r = 0.74 \pm 0.06$; mean $\pm$ S.D, six cells from six seedlings and three-independent experiments). However, in contrast to GFP-CC1, the migration of GFP-CC1YYAA was largely independent of cortical microtubules (mCherry (mCh)-TUA531; Fig. 5d–f). This indicates that reduced microtubule binding of GFP-CC1YYAA either directly affects the ability of CSCs to engage with microtubules, or that the microtubule array is mis-regulated and cannot fulfill its guiding function anymore.

Mutation of CC1 disrupts salt tolerance. To investigate whether the CC1YYAA can sustain microtubule and CSC function during salt exposure, we exposed seedlings to 200 mM salt and recorded time series of microtubule (mCh-TUA5) and CC1 (GFP-CC1 or GFP-CC1YYAA) behavior (Supplementary Fig. 7f). The GFP-CC1 proteins (either GFP-CC1 or GFP-CC1YYAA) were considered as proxy for the CSC behaviour because they co-localize and migrate together with tdt-CESA6. In agreement with5, the
Fig. 4 The N-terminus of CC1 binds to taxol-stabilized microtubules via short hydrophobic and conserved regions. 

a) $^1$H-$^{15}$N HSQC spectrum of free CC1ΔC223 (black) and in the presence of equimolar taxol-stabilized microtubules (green). Selected residues are labeled. b) F$_2$-cross sections, showing $^1$H-signals, taken along dotted lines in a at $^{15}$N frequencies 122.9 and 124.7 ppm. c) Intensity ratio of free CC1ΔC223 HNCA signals and in complex with microtubules. Minima are highlighted with green bars. Site-specific evolutionary conservation calculated by CONSURF is plotted above the sequence in a color code (green = conserved, red = unconserved). d) Hydrophobicity scores of CC1ΔC223 according to the Kyte-Doolittle scale, calculated in a 5-residue window. e) Hydrophobicity scores of Tau(201–320) according to the Kyte-Doolittle scale, calculated in a 5-residue window. Sequence conservation is plotted above the sequence like in c. Green bars highlight the interacting regions of Tau with microtubules as in ref. 29. f) Intensity ratio of free CC1YYAAΔC223 HNCA signals and in complex with microtubules. Mutated N-terminal region highlighted with blue bar.
microtubule array and cellulose synthesis were restored within 28 h of salt exposure in the GFP-CC1-complemented cc1cc2 seedlings (Fig. 5g). However, the cc1cc2 GFP-CC1YYAA-complemented seedlings largely mimicked the cc1cc2 mutant seedlings and failed to restore the microtubule array and cellulose synthesis during the course of the experiment (Fig. 5g). Interestingly, while the cc1cc2 GFP-CC1 line showed increased microtubule bundling of the salt-adjusted microtubule array, the cc1cc2 GFP-CC1YYAA cells failed to do so (Fig. 5h). Furthermore, the microtubule dynamics differed in the GFP-CC1 and GFP-CC1YYAA cell lines (Supplementary Fig. 7g-h), indicating that the microtubule dynamics and bundling are key to build a salt-tolerant microtubule array. Hence, the YY-containing region of CCI is necessary to sustain microtubule array organization and cellulose synthesis during salt stress.

Discussion
Abiotic stress, such as soil salinity, substantially impacts plant growth and thus dramatically compromises global agricultural...
productivity (~50–80% loss in yield\textsuperscript{[33,34]}). Unraveling molecular mechanisms that can be used to engineer plants for better stress tolerance is therefore of urgent importance. A potential target is the CC protein family that enables plants to sustain cellulose synthesis and the integrity of the cortical microtubule array during salt exposure\textsuperscript{5}. Here, we describe how CC1 mediates the formation of a stress-stable cortical microtubule array. CC1 contains four motifs that transiently engage with microtubules and that enable microtubule polymerization and bundling, which facilitate microtubule reassembly after stress. The hydrophobic interactions of the CC1-microtubule complex could permit a more robust binding under conditions of high-ionic strength, corroborating the importance of the protein’s function during salt stress. The two tyrosine residues in the most N-terminal microtubule-binding region of CC1 are key to the microtubule binding, both in vitro and in vivo. Mutations in these residues disrupted microtubule-guided CSC movement and led to failure in the generation of a stress-tolerant microtubule array.

Our results show that the microtubule binding characteristics of CC1\textsubscript{AC}223 are remarkably similar to that of Tau, while there is a characteristically different overall sequence architecture (Fig. 6a). Both Tau and CC1\textsubscript{AC}223 are intrinsically disordered proteins that can diffuse bidirectionally along the microtubule lattice\textsuperscript{[10,29]}. While the typical PGGG-containing repeats of the Tau microtubule-binding domain (R1–R4) are not obvious from the CC1 sequence, the two proteins do contain four similarly spaced hydrophobic microtubule-binding regions (regions 1–4 in Fig. 6a, top). A sequence comparison of these four regions (Fig. 6a, bottom) reveals a surprisingly high number of identical or similar residues, implying evolutionary convergence of the microtubule-binding mechanism. A Tau fragment encompassing the four NMR-derived microtubule-binding regions (Tau (208–324); TauF4) joins microtubules wall-to-wall similar to that of CC1\textsubscript{AC}223\textsuperscript{335}. In-depth NMR studies using TauF4\textsuperscript{346}, and cryo-EM studies on full-length Tau\textsuperscript{8}, proposed that microtubule-bound Tau spans multiple tubulin heterodimers along the microtubule principal axis. The equivalence of cross-linked positions on \(\alpha\)-tubulin\textsuperscript{22} between CC1\textsubscript{AC}223 and Tau and the longitudinal microtubule decoration of CC1\textsubscript{AC}223 in the gold-labeling experiments could suggest a similar interaction of CC1\textsubscript{AC}223 with microtubules. Comparable to the effects of tyrosine to alanine mutations in CCI, disease-related mutations in Tau cause distinct defects in microtubule organization\textsuperscript{37}. Furthermore, Tau-depleted rat neurons exhibit a reduction of microtubule dynamics\textsuperscript{38}, similar to what we observed for the mis-regulated microtubule array in both the cc1\textsubscript{AC} knockout\textsuperscript{2} and the CCI-YYAA complemented mutant. Further functional and structural analogies between Tau and CC1 are reflected in the fact that both Tau and CC1 are relevant for the organism to function during stress conditions; CC1 promotes cellulose synthesis during salt stress\textsuperscript{2}, whereas, Tau has emerged as a key regulator of stress-induced brain pathology in mice and oxidative stress in cultured fibroblasts\textsuperscript{39,40}.

While there are functional and structural analogies between CC1 and Tau, other features of the two proteins are clearly different. For example, CC1 contains a putative transmembrane and an apoplastic domain\textsuperscript{5}, whereas Tau is a cytoplasmic protein with a N-terminal projection domain that regulates microtubule spacing (Fig. 6a)\textsuperscript{31}. Moreover, CC1 is a core component of the CSC, which is primarily localized on bundled cortical microtubules and its movement is guided by cortical microtubules in plant interphase cells (Fig. 6b)\textsuperscript{3}. In this setting, the CC1 microtubule-binding regions interact with tubulin dimers in one, or multiple microtubules, and the microtubule-binding motif that contains the two tyrosine residues essential for stress-stable microtubule array formation is most distal to the plasma membrane. Given the local environment of CC1, i.e. being part of the CSC and integral to the plasma membrane, this distal motif might be the most prominently exposed of the four microtubule-binding motifs and therefore also most prominent in the microtubule engagement. Notably, the microtubule arrays have different design principles in animal and plant cells. The centrosome-coordinated microtubules in animal cells typically radiate from the cell center towards the periphery, while growing plant cells have a cortical microtubule array, with evenly distributed microtubules along the depicted yellow line. Note that the GFP signal does not substantially correlate with the mCherry signal. f Quantification of GFP-CC1 and GFP-CC1YYAA fluorescent foci on cortical microtubules in a 50 × 50 pixel area of five individual time-lapse images, \(n = 5\) cells from 5 seedling and three independent experiments (box plots: Center lines show the medians; box limits indicate the 25th and 75th percentiles; whiskers extend to the minimum and maximum). Welch’s unpaired t-test; ***\(p \leq 0.001\). g Quantification of microtubule and GFP-CC (GFP-CC1 or GFP-CC1YYAA) coverage at the cell cortex and plasma membrane, respectively, after exposure of cc1\textsubscript{AC}223 seedlings to 200 mM NaCl as in an experiment shown in Supplementary Fig. 7f. Time indicates time after salt exposure. Values are mean ± S.E.M., \(n = 27\) cells from 3 seedlings per time point and three-independent experiments. Two-way ANOVA analysis of microtubule coverage: \(p \leq 0.001\) (genotype), \(p \leq 0.001\) (time), \(p \leq 0.001\) (genotype × time). Two-way ANOVA analysis of GFP-CC protein density; \(p \leq 0.001\) (genotype), \(p \leq 0.001\) (time), \(p \leq 0.001\) (genotype × time). h Quantification of microtubule bundling after exposure of cc1\textsubscript{AC}223 GFP-CC1/GFP-CC1YYAA seedlings to 200 mM NaCl as in an experiment shown in Supplementary Fig. 7f. The salt-adjusted microtubule array in GFPCC1 seedings shows increased bundling after exposure to salt while the array GFPCC1YYAA seedlings does not. Dots represent individual data points of the corresponding bars. Values are mean ± S.E.M., \(n = 27\) cells from 3 seedlings per time point and three-independent experiments. Two-way ANOVA analysis of microtubule bundling (excluding T2 and T28); \(p \leq 0.001\) (genotype), \(p \leq 0.001\) (time), \(p \leq 0.001\) (genotype × time).
The two overlapping PCR products were purified and subsequently inserted into the linearized vector backbone using the Gibson cloning method \cite{43}. The resulting mutated pENTR-CC1YYtoAA was subsequently inserted into UBNGFPpDEST \cite{44,45} by performing LR reactions of the Gateway cloning system (Invitrogen, USA).

For heterologous protein expression of CC1ΔC223 with a hexa histidine-tag in *E. coli*, the construct pDEST17CC1ΔC223 was used \cite{5}. To obtain the hexa histidine-tagged, mutated 26YY27 to 26AA27 version of CC1ΔC223, pENTRCC1ΔC223 was linearized with Apal and BamH1. Two mutation PCRs were performed using pENTRCC1ΔC223 as a template and the primers "mut general rev", "YY to AA fw" and "mut general rev" as described above. The two overlapping PCR products were purified and subsequently inserted into the linearized vector backbone using the Gibson cloning method. The resulting pENTR-CC1ΔC223YYAA was subsequently inserted into the Gateway™ pDEST™17 Vector (Invitrogen, USA) by performing LR reactions.

For the production of NMR-samples from an ineffective pETM11 His_Sumo_CC1ΔC223 construct the Sumo part was removed and a 3C site was introduced. That construct and two mutants were made by a modified QuickChange protocol, introducing mutation sites via primer amplification (primers 14–17; Supplementary Table 1) of the whole vector by KOD-polymerase (Novagen, Merck, Germany) and subsequent digest of parental vector by DpnI (fast digest, Thermo Fisher Scientific Inc., USA). The setup was transformed into Giga cells (Novagen, Merck, Germany) for plasmid preparation and sequencing (Source Bioscience, UK).

**Representative image acquisition of seedlings.** Representative images of seedlings grown on plates with a diversity of drugs were acquired with a Leica M205 FA or Zeiss AxioZoom.V16 microscope. Before imaging, the seedlings were transferred to fresh MS plates. The staining function of Leica LAS X Life Science software was used when seedling did not fit into one image at lowest magnification.
**Heterologous protein expression.** 6XHis-tagged CC1ΔC223 and 6XHis-tagged CC1ΔC223YYAA (using the Gateway® pDEST™17 Vector (Invitrogen, USA) were applied with the following gradient: 3% B to 12% B for 1 min, 12% B to 35% B in 2 min. The samples were imaged on a Philips CM120 BioTWIN transmission electron microscope equipped with a Gatan MultiScan 791 CCD camera and a tungsten filament at an accelerating voltage of 120 kV. Microtubule bundling was assessed in images by counting the number of microtubules per bundle (degree of bundling) and the percentage of microtubules in images that were incorporated into bundles.

**Gold labeling and TEM of microtubule-associated CC1.** Microtubules were polymerized in the presence of CC1 as described above, but the pelletted microtubules were resuspended in BRBB0 without EGTA plus 5 µM taxol. 5 nm gold functionalized with a Ni-NTA group (NanoVesicles #2002) was added to the CC1-C223ΔYYAA mixture to a final concentration of 10 nM at room temperature. To remove unbound gold, the CC1-microtubule mixture was applied to a 3 k spin column (Pall Naonsep) and centrifuged in 30 s intervals at 3000 × g at room temperature. The concentration of the labeled protein was calculated using the following equation: $\text{conc} = \frac{\text{Abs} \times \text{dilution factor}}{\text{Abs} \times \text{dilution factor} + \text{CF488A}}$, where Abs is the absorbance at 488 nm and CF488A is the molar extinction coefficient of 6XHis-CC1ΔC223 in mg/ml = 0.803; df = dilution factor.

**Fluorescent labeling of His-CC1ΔC223.** Freshly purified 6XHis-CC1ΔC223 was expressed in 50 mM Tris-HCl (pH 7.25), 200 mM NaCl at a concentration of 60 µM was used (see above for purification details). CF488A CF Dye Maleimide (Biotium, Inc., Hayward, CA) was conjugated to the C-terminus of 6XHis-CC1ΔC223 in 50 mM Tris-HCl (pH 7.4), 200 mM NaCl the concentration of 60 µM was used. Samples were imaged on a Tecnai G2 Spirit TEM equipped with an FEI Eagle 4k-HS CCD camera and a LaB6 filament at an accelerating voltage of 120 kV. Microtubule bundling was assessed in images by counting the number of microtubules per bundle (degree of bundling) and the percentage of microtubules in images that were incorporated into bundles.

**Cross-linking and tandem mass spectrometry.** 6XHis-CC1ΔC223 was purified as described above but 50 mM Tris-HCl (pH 7.4) was exchanged for 50 mM sodium phosphate buffer (pH 7.0). The protein was diluted in the same buffer to a concentration of 1 mg/ml. Tubulin was solubilized to a final concentration of 1 mg/ml in 50 mM sodium phosphate buffer (pH 7.0). 6XHis-CC1ΔC223 and tubulin were mixed with 28 µl of 50 mM sodium phosphate buffer (pH 7.0). The reaction was incubated at 4 °C for 2 h. Free EDC was removed by gel filtration using PD-10 Desalting Columns (GE Healthcare, USA) according to the protocol in the manual. The column was equilibrated with 50 mM Tris-HCl (pH 7.4), 200 mM NaCl. In a final step, proteins were concentrated at 12 °C and 3000 × g at room temperature for 30 min, and supernatant was discarded. Microtubules were resuspended in 100 µl BRBB0 with 5 µM taxol. 10 µl of sample was applied to copper grids (Gilder) coated with 0.3% formvar (Electron Microscopy Sciences) and absorbed for 30 s, washed once quickly with dH2O, and stained with 10 ml of 1% aqueous uranyl acetate solution for 60 s, rinsed once quickly with dH2O, and dried. Samples were imaged on a Philips CM120 BioTWIN transmission electron microscope equipped with a Gatan Multiscan 791 CCD camera and a tungsten filament at an accelerating voltage of 120 kV or a Tecnai G2 Spirit TEM equipped with an FEI Eagle 4k-HS CCD camera and a LaB6 filament at an accelerating voltage of 120 kV. Microtubule bundling was assessed in images by counting the number of microtubules per bundle (degree of bundling) and the percentage of microtubules in images that were incorporated into bundles.
The degree of labeling (DOL) was calculated using the following equation: 

\[ \text{DOL} = \frac{A_{490}}{A_{280} \times \text{dilution factor}} \]

where 490 nm and 280 nm represent absorbance at two wavelengths used to measure the concentrations of the samples. The dilution factor was determined by comparing the absorbance at 280 nm of the sample before and after dilution with buffer.

**Microtubule diffusion assay.** Flow cells were made from two glass cover slips and 1 mm wide stripes of double-sided sticky tape (tesa, Germany). General handling was performed as described earlier. Briefly, Rhodamine-labeled tubulin (Cytoskeleton, Inc., USA) was mixed in a 1:20 ratio with unlabeled tubulin (Cytoskette, Inc., USA). 4 mg/ml of the mixture were polymerized to microtubules, stabilized with taxol and bound onto an anti-β-tubulin antibody (1:200 dilution; Sigma Monoclonal Anti-β-Tubulin #T7816) coated imaging channel as described earlier. CF488A-labeled CC1 was then mixed with the imaging channel and incubated for 30 s. Unbound protein was washed out with antifade solution. To image the motility of CC1 proteins, objective-type total internal reflection fluorescence (TIRF) microscopy was carried out on an inverted AxioObserver equipped with a TIRF slider system (both from Zeiss, Göttingen, Germany). The slider was fiber-coupled to a 488 nm diode laser (Stradus 488-50, Vortran Laser Technology, Sacramento, CA) and a 532 nm diode-pumped solid-state laser (Cobolt Samba, 100 mW, Cobolt, Stockholm, Sweden). The microscope was equipped with a Lumen 200 metal arc lamp (Prior Scientific Instruments, Jena, Germany) to provide fluorescence excitation in epi-illumination. Excitation and detection of fluorescence was achieved using a ×63, NA1.46 alpha-Plan-Apochromat oil immersion objective from Zeiss. Fluorescence filters: Unless otherwise mentioned we used the following filters from Semrock (Fluorview): (1) excitation filter for C223 proteins, objective-type total internal reflection fluorescence (TIRF) recording mode (10 frames per s) of the MetaMorph imaging software (Molecular Devices, Sunnyvale, CA).

The recorded movies of the in vitro assays were analysed using the open-source software Fiji34 and FIESTA.36 Movies were background subtracted and frame-registered using standard plugins of Fiji. The intensity of microtubules and CC1 was measured using a customized plugin recording mode (10 frames per s) of MetaMorph imaging software (Molecular Devices, Sunnyvale, CA).

The concentration of tubulin ranged from 10 to 40 µM and taxol was added in equimolar concentration. The sample was incubated at 37 °C for 45 min and fractionated by ultracentrifugation at 100,000 × g for 1 h. For NMR experiments the pellet was resuspended in a 1:1 mixture of the microtubule assembly buffer and 50 mM Tris-HCl (pH 7.4), 200 mM NaCl. Electron microscopy showed that the microtubules remained stable during the course of the experiments.

**NMR spectroscopy and data analysis.** All experiments were performed at 20 °C and 600 and 750 MHz Bruker Avance spectrometers (Bruker, Karlsruhe, Germany) equipped with cryogenically cooled triple resonance probe heads on samples containing 10% D2O in the above mentioned buffer ratio. The raw NMR data were collected, processed and exported using the Topspin software (Bruker, Karlsruhe, Germany).

Free 15N,13C-labeled CCA1C223 was assigned using three-dimensional HNCA, HC(C)ACB and four-dimensional HNCOCA and HNCA CO experiments. The two 3D experiments were acquired each with 512 × 44 × 64 complex data points in the direct F1 (H) and the two indirect F2 (15N), F3 (13C) dimensions resulting in 51 ms, 22 ms and 6.4 ms of acquisition time, respectively. The 4D data were acquired using non-uniform-sampling with 22% sparse sampling and reconstructed using the MDD routine implemented in the Bruker TopSpin processing software. All spectra were recorded as BEST-type experiments on a sample with a 250 µM concentration. Intensity ratios were calculated based on BEST-type HNCA CO and HNCA CO (150 µM) spectra recorded at 300 K. The 15N HNCA CO and the 1H-HNCA CO spectra were acquired with a mixing time of 20 ms and a 10 Hz line broadening. The intensity of doublets were joined by another single microtubule. At best, we could differentiate microtubules. Next we measured the intensity of regions where two single microtubules were joined. For this purpose, we counted by starting out measuring the intensity of clearly visible single microtubules. Each experiment was acquired with 16 scans and 512 × 36 × 44 complex data points, corresponding to an acquisition time of 69 ms in the 1H (direct), F2 and F3 dimensions. The acquisition time of the other dimension was 51 ms, 22 ms and 6.4 ms for the 15N and 13C, respectively. The total measurement time of each experiment was 20 h. The chemical shift analysis for the detection of structure propensity utilized the random-coil chemical shift library ncIDP.38 The chemical shifts were automatically referenced using the method described by Marsh et al.30

The 15N-2, 15N-3 and CPMG relaxation dispersion measurements were carried out using a 1H, 15N HSQC-based experiment which was recorded as a pseudo 3D with single-FID interleaving and WALTZ 16 15N-decoupling during acquisition periods. Each 2D plane was comprising 512 × 128 complex data points in the 1H (direct, F2) and 15N (indirect, F3) dimensions. To 15N-2, 15N-3 and CPMG relaxation dispersion experiments were measured at 54°C, 25°C, 20°C, 15°C, 10°C and 5°C. The samples were incubated for 30 min at RT to pellet microtubules and bound proteins. Supernatant and pellet fractions were subjected to SDS-PAGE and protein levels in both supernatant and pellet fractions were measured using a Coomassie stain.

**Circular dichroism spectroscopy.** 6xHis-CC1C223 was dialyzed against pure water overnight at 4 °C. The sample was spun down at 20,000 × g for 10 min, the supernatant consisting of soluble protein was diluted to 0.1 mg/ml and transferred into a 0.1 ml path length cuvette. The background was recorded with pure water. Circular dichroism (CD) spectra were recorded at room temperature (approx. 22 °C) on a Jasco-715 spectropolarimeter (Jasco Analytical Instruments, USA) equipped with a 0.01 mm path length cuvette. The background was recorded with pure water. The CD signal of the sample was recorded at 340 nm with a scan rate of 100 nm/min and an acquisition time of 3 s. When the data reached a plateau, the data was averaged and the first and second derivatives were calculated. The CD scan was repeated three times and the data was averaged.

**Sequence conservation.** Tau and CC1 evolutionary sequence conservation was calculated by BLAST against the closest homologous sequences identified by BLAST for each protein.83,93

**Analytical ultracentrifugation.** Sedimentation velocity (SV) experiments were performed using a Beckman Optima XL-A analytical ultracentrifuge. Two-channel centerpieces were loaded with 400 µl samples of CC1C223 in dialysis buffer at concentrations ranging from 15 to 60 µM. SV runs were performed between 100,000 × g at 20 °C. Absorbance scans at 280 nm were collected every 5 min. Sedimentation coefficient distributions s(s) were determined using the program Sedfit.64 The partial-specific volume of CC1C223 was predicted as 0.709 ml/g based on its amino acid sequence using the program PDB-PROTEIN. Figures were created with GUSI (available at http://biophysics.swmed.edu/MRB/software.html).
fractions were analyzed using the Gel-function of Fiji. Final dissociation constant (K_d) was estimated by fitting a saturation binding curve onto the data points with GraphPad Prism v8.0 (GraphPad software, Inc., USA).

Cell wall analysis. To quantify the cellulose content under salt stress, wild-type and mutant seedlings were grown on control plates (MS media) for 2 days and then transferred to plates containing 150 mM NaCl. After 5 more days of growth, the seed coats were removed and crystalline cellulose was quantified as before.

Live cell-imaging and data processing. XFP-tagged proteins were imaged with a CSU-W1 Yokogawa spinning disc head fitted to a Nikon Eclipse Ti-E inverted microscope with a CFI PlanApo ×100 N.A. 1.40 oil immersion objective, an EM-CCD ImageEM IC (C9100-14) (Hamamatsu Photonics, Japan), and a ×1.2 lens between the spinning disc and camera. GFP was imaged using a 488 nm solid-state diode laser. Microtubule fluorescence was then calculated by dividing the nuclear diode laser and a 609/54 nm emission filter. Time lapse images were processed and analyzed with Fiji. Drifts were corrected by using the plugin StackReg or MultStackReg in cases where two channels were imaged.

When the drift of samples could not be removed in this way, they were excluded from the analysis. Backgrounds were subtracted by the "Subtract Background" tool (rolling ball radius, 30–50 pixels). To quantify CC velocities three frames were averaged by "WalkingAverage" and kymograph analysis was performed with the kymograph tool of FIESTA. Co-localization analysis was performed using the JACoP plugin of Fiji. For the dual-labeled lines GFP-CC1/GFP-CC1YAA and tubulin RFP-Cos6 the van Steensel’s cross-correlation function (CCF) was determined by shifting one channel in x-direction pixel per pixel relative to the corresponding channel and calculating the Pearson coefficient. Correlation maxima at shift = 0 pixels indicates co-localization of both channels for most fluorescent foci.

Microtubule quantification and particle detection. Microtubule and particle density at the plasma membrane of cells were determined as described before. Briefly, cortical microtubules were quantified using a Matlab (The MathWorks, Natick, USA) based program, which applied a Sobel edge-detection algorithm at various detection thresholds to raw microscopy images. Detection thresholds were determined manually by choosing a threshold that chose most microtubules and least noise pixels. The cell area was subsequently determined by applying expansion and closing steps to the detected edge-pixels and led to enclosed microtubule regions. Microtubule coverage was then calculated by dividing the calculated area by the cell area. By using a self-written Fiji macro, particle density at the plasma membrane was determined. The cell area was detected by convoluting a wide Gaussian kernel (sigma = 1.33 mum) into each raw image and applying an automated threshold. Particles were subsequently detected by generating a Laplacian image with Feature (Erik Meijering, Biomedical Imaging Group, EPFL Lausanne) from smoothed images (Gaussian kernel with sigma = 0.2 μm). Peaks were detected using the Find Maxima function with a noise tolerance of 800. Analogously, Golgi were detected (Gaussian kernel with sigma = 0.8 μm; noise tolerance of 120). The number of Golgi was subtracted from the number of total particles. Density was calculated by dividing the resulting particle count by the cell area.

Quantification of microtubule bundling and dynamics. Image analysis was done using Fiji. Cell boundaries were detected by convoluting a wide Gaussian kernel (sigma = 10 μm) into each image and thresholding using the Otsu algorithm. All further operations were conducted within these cell boundaries. To detect microtubules and skeletonize the raw images, a Laplacian image was generated using Feature and smoothing of 1.5 (Erik Meijering, Biomedical Imaging Group, EPFL Lausanne). A defined threshold was then applied for each individual image that covered all microtubules in the image independently of the background. The “Despeckle Filter” of Fiji was then applied. Microtubules were then detected using the “Analyze Particles” function of Fiji with a size from 20 to infinity pixels and the outside of these was cleared. The skeletonized microtubule image was then analyzed using Diameter with standard settings. The thresholded “Super mask” images were then taken as a measure for mean diameter and the following bundling of fibers. Microtubule dynamics were measured as described in detail before. Briefly, time phased image subtraction with a time shift of one frame was applied to identify shrinking and growing microtubule ends. The velocities of these growing and shrinking microtubule ends were finally analyzed with the kymograph evaluation tool of FIESTA.

Statistical analysis and experimental design. For statistical analyses, Welch’s unpaired t-test or two-way ANOVA were performed using GraphPad Prism 8. A p-value of <0.05 was considered as statistically significant. Each statistical method used to calculate p-values is defined in the corresponding figure legends. Data analysis (especially for images) was either done automatically, so independently from the investigator, or file names were removed before the analysis. For the measurement of plant size, investigators were not blinded. In this case, data were always collected according to the genotype of plants. Sample size was determined for each experiment based on similar data reported in scientific literature.

Reporting summary. Further information on experimental design is available in the Nature Research Reporting Summary linked to this article.

Data availability. All cross-linking mass spectrometry data are available at PRIDE with the identifier PXD009260. The NMR chemical shift data are deposited in the BMRB database with accession number 27660. Data underlying Fig. 1a, f-h, Fig. 2b, e–d, Fig. 4c–f, Fig. 5a, c–e, h, Fig. 5a, Fig. S1a, Fig. S1b, c, e, f, Fig. S4a-g, Fig. 5a–e, g, Fig. S6j, Fig. S7d–e, g-h are provided as a Source Data file. Any other data are available from the corresponding authors upon request.

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References
1. Roostalu, J. & Surrey, T. Microtubule nucleation: beyond the template. Nat. Rev. Mol. Cell Biol. 18, 702–710 (2017).
2. Mach, I. Plant cortical microtubules array recruitment mechanisms in common with centrosomes. Plant Cell 24, 2 (2012).
3. Pardeed, A. R., Somerville, C. R. & Ehrlhardt, D. W. Visualization of cellulose synthesis demonstrates functional association with microtubules. Science 312, 1491–1495 (2006).
4. McFarlane, H. E., Döring, A. & Persson, S. The cell biology of cellulose synthesis. Annu. Rev. Plant. Biol. 65, 69–94 (2014).
5. Endler, A. et al. A mechanism for sustained cellulose synthesis during salt stress. Cell 162, 1353–1364 (2015).
6. Ballatore, C., Lee, V. M.-Y. & Trojanowski, J. Q. Tau-mediated neurodegeneration in Alzheimer’s disease and related disorders. Nat. Rev. Neurosci. 8, 663–672 (2007).
7. Arendt, T., Steier, J. T. & Holzer, M. Tau and tauopathies. Brain Res. Bull. 126, 238–292 (2016).
8. Kellogg, E. H. et al. Near-atomic model of microtubule-tau interactions. Science 360, 1242–1246 (2018).
9. Dreechel, D. N., Hyman, A. A., Cobb, M. H. & Kirschner, M. W. Modulation of the dynamic instability of tubulin assembly by the microtubule-associated protein tau. Mol. Biol. Cell 3, 1141–1154 (1992).
10. Hinrichs, M. H. et al. Tau protein diffuses through the microtubule lattice. J. Biol. Chem. 287, 38559–38568 (2012).
11. Scott, C. W., Kilia, A. B., Lo, M. M. S., Norris, T. E. & Caputo, C. B. Tau protein induces bundling of microtubules in vitro: comparison of different tau isoforms and a tau protein fragment. J. Neurosci. Res. 33, 19–29 (1992).
12. Drubin, D. G. & Kirschner, M. W. Tau protein function in living cells. J. Cell Biol. 103, 2739–2746 (1986).
13. Trojanowski, J. Q., Schuck, T., Schmidt, M. L. & Lee, V. M. Distribution of tau proteins in the normal human central and peripheral nervous system. J. Histochem. Cytochem. 37, 209–215 (1989).
14. Mucke, L. Neuroscience: Alzheimer’s disease. Nature 461, 895–897 (2009).
15. Gardiner, J., Overall, R. & Marc, J. Distant plant homologues: don’t throw out the baby. Trends Plant. Sci. 17, 126–128 (2012).
16. Gardiner, J. The evolution and diversification of plant microtubule-associated proteins. Plant J. 75, 219–229 (2013).
17. Rappasibn, J. The beginning of a beautiful friendship: cross-linking/mass spectrometry and modelling of proteins and multi-protein complexes. J. Struct. Biol. 173, 530–540 (2011).
18. Götz, M. et al. StavroX-A software for analyzing crosslinked products in protein interaction studies. J. Am. Soc. Mass Spectrom. 23, 76–87 (2012).
19. Yang, B. et al. Identification of cross-linked peptides from complex samples. Nat. Methods 9, 904–906 (2012).
20. Lima, D. B. et al. SIM-XL: a powerful and user-friendly tool for peptide cross-linking analysis. J. Proteom. 129, 53–55 (2015).
21. Park, C. Y., Klammers, A. A., Käll, L., MacCoss, M. J. & Noble, W. S. Rapid and accurate peptide identification from tandem mass spectra. J. Proteome Res. 7, 3022–3027 (2008).
22. Kadavath, H. et al. Tau stabilizes microtubules by binding at the interface between tubulin heterodimers. Proc. Natl Acad. Sci. USA 112, 7501–7506 (2015).
23. Gigant, B. et al. Structural basis for the regulation of tubulin by vinblastine. Nature 435, 519–522 (2005).
24. Ravelli, R. B. G. et al. Insight into tubulin regulation from a complex with colchicine and a stathmin-like domain. Nature 428, 198–202 (2004).
25. Reddy, V., Lymar, E., Hu, M. & Hainfeld, J. 5 nm Gold-Ni-NTA binds His tags. Microsc. Microanal. 11, 1118CD (2005).
26. Gell, C. et al. Microtubule dynamics reconstituted in vitro and imaged by single-molecule fluorescence microscopy. Methods Cell Biol. 95, 221-245 (2009).

27. Tamiola, K. & Mulder, F. A. A. Using NMR chemical shifts to calculate the propensity for structural order and disorder in proteins. Biochem. Soc. Trans. 40, 1014-1020 (2012).

28. Tamiola, K., Acar, B. & Mulder, F. A. A. Sequence-specific random coil chemical shifts and locally disordered regions. J. Am. Chem. Soc. 132, 18000-18003 (2010).

29. Mukrasch, M. D. et al. Structural polymorphism of 441-residue tau at single residue resolution. PLoS Biol. 7, e34 (2009).

30. Sampaikumar, A. et al. Patterning and lifetime of plasma membrane-localized cellulose synthase is dependent on actin organization in Arabidopsis strobilus cells. Plant Physiol. 162, 675-688 (2013).

31. Gutierrez, R., Lindelbohm, J. J., Paredez, A. R., Emons, A. M. C. & Ehrtwardt, D. W. Arabidopsis cortical microtubules position cellulose synthase delivery to the plasma membrane and interact with cellulose synthase trafficking compartaments. Nat. Cell Biol. 11, 797-806 (2009).

32. Cramer, G. R., Urano, K., Dérot, S., Pezzotti, M. & Shinozaki, K. Effects of abscisic acid on plants: a systems biology perspective. BMC Plant Biol. 11, 163 (2011).

33. Shrivastava, P. & Kumar, R. Soil salinity: a serious environmental issue and plant growth promoting bacteria as one of the tools for its alleviation. J. Environ. Biol. 36, 131 (2015).

34. Laemmli, U. K. Denaturing (SDS) discontinuous gel electrophoresis. Nature 277, 680-685 (1979).

35. Shevchenko, A., Thomas, H., Havlis, J., Olsen, J. V. & Mann, M. In-gel digestion for mass spectrometric characterization of proteins and proteomes. Nat. Protoc. 1, 2856-2860 (2006).

36. Kauppinen, R., Eichler, A., Rana, S. M., Østergaard, J. F., Brosius, J. F. & Mann, M. A protein identification and analysis tool for the Expedys server. J. Proteome Res. 5, 213-222 (2006).

37. Ibañez-Salazar, A. et al. Oxidative stress modiﬁes the levels and phosphorylation state of Tau protein in human fibroblasts. Front. Neurosci. 11, 495 (2017).

38. Chen, J., Kanai, Y., Cowan, N. J. & Hirokawa, N. Projection domains of MAP2 and tau determine spacings between microtubules in dendrites and axons. Nature 360, 674-677 (1992).

39. Sampathkumar, A. et al. Live cell imaging reveals structural associations between the actin and microtubule cytoskeleton in Arabidopsis. Plant Cell 23, 2302-2313 (2011).

40. Gibson, D. G. et al. Enzymatic assembly of DNA molecules up to several hundred kilobases. Nat. Methods 6, 343-345 (2009).

41. Lopes, S. et al. Tau protein is essential for stress-induced brain pathology. Proc. Natl. Acad. Sci. USA 113, E3575-E3576 (2016).

42. Ibáñez-Salazar, A. et al. Oxidative stress modifies the levels and phosphorylation state of Tau protein in human fibroblasts. Front. Neurosci. 11, 495 (2017).

43. Chen, J., Kanai, Y., Cowan, N. J. & Hirokawa, N. Projection domains of MAP2 and tau determine spacings between microtubules in dendrites and axons. Nature 360, 674-677 (1992).

44. Nitzsche, B. et al. Studying kinesin motors by optical 3D-nanometry in gliding motility assays. Methods Cell Biol. 95, 247-271 (2010).

45. Schindelin, J. et al. Fiji: an open-source platform for biological-image analysis. Nat. Methods 9, 676-682 (2012).

46. Rueden, C. T. et al. ImageJ2: Image for the next generation of scientific image data. BMC Bioinfo. 18, 529 (2017).

47. Schneider, C. A., Rasband, W. S. & Eliceiri, K. W. NIH Image to ImageJ: 25 years of image analysis. Nat. Methods 9, 671-675 (2012).

48. Nitzsche, B. et al. Studying kinesin motors by optical 3D-nanometry in gliding motility assays. Methods Cell Biol. 95, 247-271 (2010).
Additional information

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