Inhibition of cell expansion by rapid ABP1-mediated auxin effect on microtubules

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The prominent and evolutionarily ancient role of the plant hormone auxin is the regulation of cell expansion1. Cell expansion requires ordered arrangement of the cytoskeleton2 but molecular mechanisms underlying its regulation by signalling molecules including auxin are unknown. Here we show in the model plant Arabidopsis thaliana that in elongating cells exogenous application of auxin or redistribution of endogenous auxin induces very rapid microtubule re-orientation from transverse to longitudinal, coherent with the inhibition of cell expansion. This fast auxin effect requires auxin binding protein 1 (ABP1) and involves a contribution of downstream signalling components such as ROP6 GTPase, ROP-interactive protein RIC1 and the microtubule-severing protein katanin. These components are required for rapid auxin- and ABP1-mediated re-orientation of microtubules to regulate cell elongation in roots and dark-grown hypocotyls as well as asymmetric growth during gravitropic responses.

Auxin is crucial for diverse developmental processes and growth responses3. One major effect of auxin is cell expansion1, which relies on the coordinated activities of cellular processes involving the cytoskeleton2. When cells elongate, cortical microtubules are arranged perpendicularly to the axis of cell elongation (transverse microtubules), whereas a longitudinal alignment accompanies growth inhibition2. The dynamic nature of microtubules provides the flexibility to rearrange them into different arrays4, enabling growth changes downstream of different signals such as gravity5 or light6. Many of these signalling pathways converge on auxin7; therefore its action upstream of microtubules translates different signals into growth responses7. Nonetheless, whether auxin acts directly on microtubule arrangement and by which mechanism remain unclear.

Microtubules co-align approximately perpendicularly to the elongation axis in roots and dark-grown hypocotyls2. The transition zone of primary root and the elongation zone of etiolated hypocotyl (Extended Data Fig. 1a) are controlled by auxin to determine their respective growth rates8. We visualized cortical microtubules in transgenic lines expressing microtubule-associated protein 4 fused with green fluorescent protein (MAP4–GFP)9 or α-tubulin 6 fused with red fluorescent protein (TU6–RFP), and classified cells on the basis of prevalent microtubule arrangement into four groups (Fig. 1a). In root, microtubules were mainly transverse and underwent visible realignment within 10 min after application of the synthetic auxin naphthalene-1-acetic acid (NAA), leading to partial longitudinal re-orientation after 1 h (Fig. 1a). Comparable effects were observed irrespective of the microtubule reporter following treatment with the natural auxin indole-3-acetic acid (IAA) (Extended Data Fig. 1b–f). The same effects were observed in etiolated hypocotyls (Fig. 1b) although at a higher auxin concentration, consistent with known auxin response maxima of aerial tissues at higher doses10. Re-orientation of microtubules is not always homogenous, as revealed by the deviated angle of individual microtubules. Transverse microtubules (90°) decreased at the expense of increasingly oblique and longitudinal microtubules (0−60°/120°−180°) following auxin treatment (Extended Data Fig. 1c, f).

Treatment with the weak auxin analogue11 of 2-NAA showed a very weak effect on microtubule rearrangement whereas acidic pH led to massive disruption and random orientations of microtubules (Extended Data Fig. 1d, e).
DATA Fig. 1g, h). Both treatments confirm the specificity of active auxins on microtubule orientation.

In roots, gravistimulation induces asymmetric auxin redistribution: lower levels at the upper side correlate with cell elongation and higher levels at the lower side with inhibition of cell expansion17. We assessed the effect of endogenous auxin redistribution on microtubule re-arrangement by tracking trajectories of end binding 1b (EB1b), a protein that preferentially accumulates at the growing plus ends of microtubules. After 90° root re-orientation, transverse microtubules were maintained in the upper-side cells whereas at the lower side, microtubules longitudinally re-oriented within 10 min, preceding growth inhibition (Fig. 1c). Auxin distribution reported by the auxin response reporter DII-VENUS14 during gravitropism confirmed higher auxin response at the lower side than the upper side (Extended Data Fig. 1i–k). Thus auxin application or endogenous auxin redistribution promotes longitudinal microtubule orientation, correlating with auxin inhibiting cell elongation.

Next we addressed the mechanism by which auxin influences microtubule orientation. The signalling pathway of the nuclear-localized auxin co-receptors transport inhibitor response 1/auxin-related F-box (TIR1/AFB)–auxin/IAA (AUX/IAA) regulates gene transcription and mediates many plant developmental effects. On the other hand, the ABP1 pathway regulates transcriptional and non-transcriptional responses such as interdigitation of pavement cells or clathrin-dependent endocytosis15.

In root cells of tir1-1/afb1-1/afb2-1/afb3-1 (ref. 18), we observed normal transverse microtubules; however, they were less sensitive to auxin treatment (Extended Data Fig. 2a, b). Functional inactivation of ABP1 in the conditional SS12S and SS12K lines resulted in microtubule orientation defects with increasing time of ABP1 inactivation (Extended Data Fig. 2c–f), whereas abp1-5, harbouring a point mutation in the auxin binding pocket susceptible to impair auxin binding, did not show altered microtubule arrangement (Fig. 2a). Nonetheless, in both cases a severe reduction in microtubule re-orientation was observed in response to auxin (Fig. 2a). Short-term ABP1 inactivation (8 h) in dark-grown hypocotyls also impaired microtubule responsiveness to auxin without affecting the microtubule organization (Fig. 2b). This observation, together with a similar effect of abp1-5 mutation, confirms that the microtubule insensitivity to auxin does not result from pre-existing microtubule alteration in these lines. Growth of ABP1-inactivated roots was previously reported to be auxin resistant, strengthening the correlation between the effect of auxin on microtubule orientation and inhibition of cell elongation.

We also explored whether ABP1 function is required for the microtubule rearrangement and differential growth response in root gravitropism. Following 90° root re-orientation, ABP1-inactivated lines showed much weaker microtubule rearrangement at the lower side than wild type (WT) (Extended Data Fig. 3a); however, gravity-induced asymmetric auxin distribution (monitored by DII-VENUS) was also less pronounced in ABP1-inactivation lines (Extended Data Fig. 3b, c compared with Extended Data Fig. 1i–k). In line with these observations, ABP1-deficient lines showed defects in gravitropic response (Extended Data Fig. 3d).

Given the mutual impact of the TIR1 and ABP1 pathways, it is difficult to distinguish between direct and indirect effects; however, because short-term ABP1 inactivation strongly impairs auxin-mediated microtubule re-orientation, this favours the ABP1 pathway as the primary mechanism. To gain further mechanistic insights into the effect of auxin on microtubule and transcriptional compared with fast, non-transcriptional responses, we studied the kinetics of auxin’s effect on microtubule re-orientations by following EB1b movement at 15 s intervals, and its trajectories. NAA did not influence the speed of EB1b movement (Extended Data Fig. 4a, b) but gradually increased oblique and longitudinal EB1b tracks (0–60°/120–180°) after 75 s (Fig. 3a and Supplementary Videos 1 and 2). To provide quantitative measures of...
microtubule rearrangements, we developed an image analysis tool that automatically assigned EB1b trajectories to transverse (depicted as blue area) or longitudinal (red area) directions, which revealed changes in EB1b track orientations as soon as 30 s after auxin treatment (Extended Data Fig. 4c). Both types of kinetic analysis revealed a very fast responsiveness of microtubules to auxin, making transcriptional regulation in this process unlikely. This is consistent with the lack of interference on auxin-induced microtubule rearrangement by blocking transcription with cordecypin (Extended Data Fig. 4d). Involvement of the ABP1-dependent response pathway is also supported by the inhibition of the TIR1/AFB pathway with α-(phenylethyl-2-one)-IAA (PEO-IAA)\textsuperscript{21}, which did not prevent the effect of IAA on microtubules (Extended Data Fig. 4e).

To investigate further the rapid ABP1-dependent effect on microtubules, we introduced EB1b–GFP in ABP1-inactivated lines. Trajectories of EB1b following ABP1 inactivation showed more oblique and longitudinal microtubules than WT (Fig. 3b, Extended Data Fig. 4f–i and Supplementary Videos 3 and 4). After NAA treatment, no consistent switch of EB1b trajectories to longitudinal directions, but only a few stochastic changes, were observed (Supplementary Videos 5 and 6, Fig. 3b and Extended Data Fig. 4f–i compared with WT in Fig. 3a and Extended Data Fig. 4c). By a complementary approach, we analysed the effect of auxin on EB1b trajectories in inducible ABP1 gain-of-function lines (XVE ≥ ABP1-OE). Overexpression of ABP1–GFP in the presence of oestradiol increased the overall amount of ABP1 (Extended Data Fig. 5a–c), leading to an enhanced microtubule re-orientation in response to auxin (Extended Data Fig. 5d). In contrast, abp1-15 exhibited delayed microtubule re-orientation in response to auxin (Extended Data Fig. 5d). Overall these results strongly suggest that the fast, non-transcriptional effect of auxin on microtubule re-orientation is mediated primarily by ABP1-dependent signalling.

Next we addressed the downstream mechanism by which ABP1 mediates the effect of auxin on microtubule arrangement. Although auxin induces calcium transients\textsuperscript{22}, the manipulation of exogenous calcium had very different effects on microtubule arrangements compared with auxin (Extended Data Fig. 6). Then we tested the downstream components of the ABP1 pathway: the ROP6 GTPase, its effector RIC1 (ref. 16) and its downstream component microtubule-severing protein katanin (KTN1)\textsuperscript{23,24}. We analysed microtubules in rop6-1, ric1-1 and ktn1 mutants. Compared with WT, roots of rop6-1 and ric1-1 showed almost normal transverse microtubules but were much less auxin responsive (Fig. 4a and Extended Data Fig. 7a). Double mutants (SS12S ric1-1, SS12K ric1-1), with ABP1 inactivation, exhibited the SS12K/S root phenotype\textsuperscript{25} but ric1-1 microtubule arrangement (Fig. 4a and Extended Data Fig. 7a–d), consistent with the reported action of RIC1 downstream of ABP1 in early responses\textsuperscript{26}. The ktn1 mutant exhibited a severe microtubule phenotype, with completely random microtubules in roots compromising further analysis. Microtubules were less disturbed in dark-grown ktn1 hypocotyls, allowing investigation of the response to auxin and the genetic interaction with ABP1 (Extended Data Fig. 7e). Rapid auxin-induced re-orientation of microtubules was impaired in ktn1 (Fig. 4b). Inactivation of ABP1 in ktn1 (SS12K ktn1) resulted in a microtubule pattern similar to SS12K and conferred insensitivity to auxin (Fig. 4b). These data suggest that KTN1 is required for microtubule re-orientation in response to auxin but that other microtubule-associated components might be involved as well. The present data and the crucial roles of RIC1 and KTN1 in the control of microtubule architecture and crossover\textsuperscript{23,24} support the idea that auxin-dependent ABP1 signalling might act through Rho GTPases and RIC effectors on critical targets such as KTN1 for guiding microtubule orientation.

The effects of auxin on cell expansion are not mechanistically well understood; however, it is clear that sustainable growth control requires fast, non-transcriptional auxin effects and regulation of transcription\textsuperscript{1}. Here we show that auxin signalling targets immediate changes in microtubule orientation. Auxin, by a non-transcriptional effect requiring ABP1 and downstream signalling, influences microtubule re-orientations within minutes, leading to changes in microtubules from transverse to longitudinal orientation to inhibit cell elongation. It is possible that branching of the ABP1 signalling for microtubule realignment occurs at the level of Rho GTPases, their RIC effectors and downstream targets such as KTN1. It remains unclear how this newly identified effect of ABP1 signalling on microtubules is related to other actions of the ABP1 pathway, such as inhibition of clathrin-mediated endocytosis, in particular given the differences in efficiencies of synthetic and natural auxins on both processes\textsuperscript{26}. The relationship between microtubules and endocytosis in plants is still unclear; however, given the observations from animals that clathrin controls microtubule acetylation\textsuperscript{27}, auxin-mediated defects in clathrin organization might cause the mis-modification of microtubules and thus disturb their crossover. On the other hand, Rho GTPases acting downstream of ABP1 might influence clathrin and

Figure 4 | Auxin–ABP1 control microtubule arrangement through downstream ROP6–RIC1 and involvement of KTN1. a, Microtubule orientation and quantification in roots of WT, rop6-1, ric1-1, SS12S/K ric1-1 following 60 min of DMSO or 100 nM NAA application. b, Microtubule orientation and quantification in 24 h ethanol-induced hypocotyls of WT, SS12K, ktn1 and SS12K ktn1 following 60 min of DMSO or 10 μM IAA application. The ratio of transverse microtubules in DMSO versus NAA/IAA treatment is indicated above the charts (a, b). In all panels, average values are shown and error bars are s.e.m. and Student’s t-test was calculated for transverse microtubules (**P < 0.001). Scale bars, 5 μm (a) and 10 μm (b).
microtubule functions via distinct effectors. Our observations on the rapid regulation of microtubule arrangement by the ABP1-mediated auxin signalling pathway provide insight into the long-sought molecular mechanism by which a major plant hormone exerts its fast effect on plant cell growth.

Online Content Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

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Supplementary Information is available in the online version of the paper.

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Author Contributions X.C., L.G., C.P.-R. and J.F. conceived the study and designed experiments. X.C. performed experiments in roots, and L.G. performed experiments in hypocotyls. H.L., S.P. and A.A. assisted in microscopy and data generation. H.R. generated partial double mutants. R.H. did bioinformatics analysis. E.B. helped with discussion of the data. X.C., L.G., C.P.-R. and J.F. wrote the manuscript.

Author Information Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to J.F. (jiri.friml@ist.ac.at) or C.P.-R. (Catherine.Rechenmann@isv.cnrs-gif.fr).
METHODS

Material and growth conditions. Seeds were sown on 0.8% agar containing 1/2 Murashige and Skoog medium with sucrose for root experiments and 1% agar containing 1/2 Murashige and Skoog medium without sucrose for hypocotyl experiments. For root analyses, seedlings were grown under 16 h light/8 h dark photoperiod; for hypocotyl analyses, seedlings were grown in darkness at 22 °C for 4 days. Ethanol induction of conditional lines for ABP1 (ref. 19) (named SS125 or SS112K) was performed by exposure of the seedlings to ethanol vapour for various times as indicated for the different assays. We routinely used 48 h of exposure to vapours generated from 500 μl of 5% ethanol in light-grown root and 8 h of exposure to vapours from 500 μl of 8% ethanol for dark-grown hypocotyls (except particular induction times annotated in the figure legends). In each single experiment, WT and ABP1 conditional lines were always grown on the same plate and exposed to ethanol in identical conditions. The root-1 and root-10 lines were in the wild-type (C58) background, the other lines were derived from the Columbia (Col-0) background. The T-DNA insertion mutant of KTN1 (SAIL_343_D12) was provided from the Arabidopsis Information Resource. Offspring of the double mutants were analysed by PCR amplification as described previously20,21. For the generation of inducible overexpressing ABP1 lines (XVE > ABP1-OE), ABP1–GFP was cloned by inserting GFP into ABP1 after the glycine 120 by primer extension PCR with two glycines flanking the GFP coding sequence2; then the fragment of full-length ABP1 genomic DNA with GFP was cloned into the Gateway vector pMDC78 using Gateway cloning technology (www.invitrogen.com). At least three independent lines were used for the analysis (including the untransformed observations). MAP–GFP, TUA6–RFP and EB1b–GFP22 were used as microtubule markers, and DII-VENUS was used as an early auxin response sensor23.

Confocal microscopy observation. For observations of microtubule orientation in root cells, in the transition zone of the primary root were visualized by vertical Zeiss LSM 700 confocal laser scanning microscope (with ×63 objective using hair gel as immersion medium). For observations in dark-grown hypocotyls, cells in the elongation zone were visualized by a Nipkow Spinning Disk confocal system (Yokogawa CXU-X1-A1) mounted on a Nikon Eclipse Ti E inverted microscope (with ×40 oil immersion objective). For dark-grown hypocotyls, all manipulations were performed under green light to avoid any light effects on microtubule re-orientation before confocal imaging. For all the visualization of auxin-treated seedlings, 1 min manipulation time was needed to apply auxin before imaging. The videos of EB1b trajectory were performed by spinning disc confocal system with ×63 water immersion objective. Videos were acquired with 300 ms exposure time every 500 ms for 10 min. The settings of excitation and detection were as follows: for GFP, 488 nm, 505–550 nm; for VENUS, 514 nm, 527 nm; for RFP, 587 nm, 610 nm. All the images in a single experiment were captured with the same setting. In experiments where rapid microtubule re-orientation was imaged, the seedlings were gently placed in chamber slides, covered by 0.8% agar containing 1/2 Murashige and Skoog medium or placed on liquid 1/2 Murashige and Skoog medium glass slides, and then placed on a vertical Zeiss LSM 700 confocal laser scanning microscope with GFP and EB1b–GFP fluorescence emission filters. As described before, the longitudinal direction parallel to and going along the growth axis was defined as 0° and the transverse direction perpendicular to the growth axis was defined as 90°. Blue strips represent the transverse direction (90 ± 30°), red strips represent the oblique and longitudinal direction (0–60°/120–180°). The measurement of deviated angles of microtubules was processed by ImageJ combined with Matlab. At least eight cells of different roots were randomly selected, and in each cell at least 100 microtubules were quantified.

Gravitropic response. Four-day-old vertically grown seedlings, under light conditions, were re-oriented by 90°, and the angles deviating from the original vertical growing direction of primary roots (defined as 0°) were tracked every 30 min until 8 h.

Quantification methods. 1. To quantify the percentage of different cell types, the number of cells at a certain microtubule orientation type was calculated as a percentage of the total measured cells in each root, and different cell types were divided into transverse, oblique, random and longitudinal groups on the basis of prevalent microtubule arrangement (Fig. 1a). At least 15 different roots were analysed and at least eight cells were quantified in each root. For hypocotyls, cells were classified into a microtubule orientation group depending on the overall angles of microtubules for each cell (0–30°/157.5°–180° for longitudinal, 22.5–67.5°/112.5–157.5° for oblique and 67.5–112.5° for transverse). Angles of microtubules were determined both manually and by using the plug-in of the ImageJ FibrilTool24. At least ten different hypocotyls were analysed and 100 hypocotyl cells were quantified.

2. To quantify the oriented angles of microtubules in root cells, the number of microtubules at a certain angle range was calculated as a percentage of the total microtubules measured. The longitudinal direction parallel to and going along the growth axis was defined as 0°, the transverse direction perpendicular to the growth axis was defined as 90°, the longitudinal direction parallel to but going opposite the growth axis was defined as 180° and angles deviating from the longitudinal direction (0°) were measured. Different microtubules angles were divided into two categories: transverse (defined as 90° ± 30°), oblique and longitudinal direction (0–60°/120–180°).

3. For the ‘time-stack’ images of EB1b trajectory (Fig. 1c), the video of EB1b trajectory was taken every 15 s per picture in a vertical Zeiss LSM 700 confocal laser scanning microscope for 10 min in total, every 3 s per picture in a spinning disc for 10 min in total. The maximum intensity projections of all frames were stacked by ImageJ (Image > Stack > Z-project). Then, the EB1b trajectory was quantified as described previously (method 2).

4. To quantify the rate of EB1b movement, the method has previously been described in details. In brief, the manual quantification of the EB1b trajectory (Fig. 3a, b) was performed according to the directions of each single EB1b track followed with strips. As described before, the longitudinal direction parallel to and going along the growth axis was defined as 0° and the transverse direction perpendicular to the growth axis was defined as 90°. Blue strips represent the transverse direction (90 ± 30°), red strips represent the oblique and longitudinal direction (0–60°/120–180°). The deviated angle of each EB1b strip from the transverse direction was measured by ImageJ. At least ten cells of different roots were randomly selected, and in each cell at least 100 EB1b tracks were quantified.

5. Additionally, automated quantification of the directionality of EB1b trajectory was used. To determine the direction of EB1b trajectory within the cell, it is necessary to eliminate the apparent motion caused by the growth of the seedling. In a first step image, stabilization was therefore performed by manually locating a prominent feature, for example the boundary between two specific cells in all frames of the video. Translating each frame accordingly then kept the prominent feature stationary. This proved more accurate than various automated image registration methods.

The temporal resolution of the videos was generally not sufficient to track individual particles over multiple frames. Thus particle tracking velocimetry resulted in a very sparse vector field. To obtain a more complete vector field that better represented the transport direction of all reporter proteins, the Horn–Schunck method of estimating optical flow was implemented and subsequently used for the analysis. In this method a regularization parameter enforces a smooth vector field which is consistent with the motion map. To obtain the vector field, the dominant direction of motion at each pixel was calculated only if the speed reached a certain threshold, which was determined by comparison with the maximum intensity projection of the video where regions with no or little protein transport appeared darker. The values chosen for the regularization parameter and the minimum speed were kept constant throughout the analysis of all videos. The direction of motion of the remaining pixels, for which the speed was above the threshold value, was sorted into two classes: blue indicates a direction of transport of 90° ± 30°; red corresponds to 0–60°/120–180°. Finally, the areal fraction of the two classes was calculated.

6. To quantify the fluorescence, DII-VENUS fluorescence was pseudo-coloured, and the epidermal and cortical cells of similar developmental stages with symmetric areas in roots were selected for quantification. The intensity of nuclei was extracted using the ROI tool of Fiji software (http://fiji.sc/wiki/index.php/Fiji), and©2014 Macmillan Publishers Limited. All rights reserved
the sum of fluorescence in the upper- and lower-side cells of each seedling was individually quantified.

**Quantitative PCR with reverse transcription.** Whole RNA of seedlings was extracted using an RNeasy Mini Kit (Qiagen) and complementary DNA was synthesized using an iScript cDNA Synthesis Kit (Bio-rad). Quantitative PCR with reverse transcription (qRT–PCR) used LightCycler 480 SYBR Green I Master (Roche) following the recommendations of the manufacturer. qRT–PCR was performed in 384-well optical reaction plates by using Perkin Elmer Janus Robot and Roche Lightcycler 480 with heat for 10 min to 95°C to activate hot-start Taq DNA polymerase, followed by 40 cycles of denaturation for 60 s at 95°C and annealing extension for 60 s at 58°C. Expression levels were normalized to the expression levels of *ACTIN8*. Specific primers of *ABP1* (F: TCGTCGTCCTTTCCGTGCG; R: TT GGCAAGCCATTGATGGGACA) and *ScFv* (F: TTACTGGATGCACTGGGTGA; R: AAGACTGACAGGCAGGGAGA) were used for gene expression as previously reported. Two biological repeats were analysed in triplicate. qRT–PCR relative quantification was performed on Lightcycler 480 software combined with the local website (http://qpcr.ista.local).

**Protein analysis.** Proteins were extracted from WT and XVE–ABP1-OE 10-day-old seedlings induced or not with oestradiol by grinding at 4°C with a mortar in the extraction buffer (50 mM Tris-HCl, pH 7.5, 1 mM EDTA, 100 μM MgCl₂, 5 mM sodium ascorbate, 500 mM sucrose, phosphatase and protease inhibitors). Samples were spun at 5,000g for 10 min at 4°C to remove cell debris. Supernatants were centrifuged at 50,000g for 60 min at 4°C to pellet the total membrane fraction. Pellets were re-suspended in microsomal buffer (25 mM Tris-HCl pH 6.8, 0.5 mM EDTA, 0.1 mM MgCl₂, 330 mM sucrose, 10% glycerol, protease inhibitor cocktail) for the following SDS-PAGE analysis. Protein loading was controlled by Coomassie brilliant blue staining. Protein gel blot used the mAb12 mouse monoclonal antibody to detect ABP1 protein. Protein amount was specified by GelQuant.NET software and normalized according to sample loading. Two biological repeats were analysed in duplicate.

**Statistics.** For all quantitative data, error bars indicate s.e.m. The number of analysed samples is indicated as *n* from at least three biological replicates, and statistical analyses used Student’s *t*-test where * or ** correspond to *P* < 0.05 or 0.001, respectively.

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Extended Data Figure 1 | Auxin induces microtubule rearrangement in root cells. a, Schematic diagram of root and dark-grown hypocotyl growth. The growth direction of the root and hypocotyl is named as the cell growth axis. The observed cells for microtubules array were in the transition zone (highlighted by red line) of roots and in the elongation zone of dark-grown hypocotyls (highlighted by grey frame). The arrays of microtubules in root and hypocotyl were depicted for the expanding cells. b–f, MAP4–GFP or TUA6–RFP visualization of microtubule orientation in roots was performed by time-lapse observation (every 10 min; prime ('), minutes) following 100 nM NAA or IAA treatment, and deviated angles of individual microtubules were quantified as transverse microtubules (90°± 30°) or longitudinal microtubules (0–60°/120–180°). In c and f, Student’s t-test was calculated for transverse microtubules compared with untreated roots (*P < 0.05; **P < 0.001). g, h, MAP4–GFP visualization and quantification of microtubule orientation in roots after 1 μM 2-NAA treatment for 60 min or after transfer of seedlings on acidified 1/2 Murashige and Skoog medium at pH 4.9 for 30, 90 and 180 minutes. Student’s t-test was calculated for transverse microtubules in treated samples compared with 1/2 Murashige and Skoog medium (pH 5.8) growing roots used as controls (**P < 0.001). i–k, Auxin distribution approximated by DII-VENUS at the lower side (LS) and upper side (US) of 90° re-oriented WT root (in DII-VENUS background). Enlarged pictures (i) are shown as the frames highlighted (k). Signal intensity is represented by the colour code as indicated. The relative signal for the upper side and lower side (j) is expressed compared with the signal in the respective frame before gravistimulation. Student’s t-test was calculated for the signal between the upper and lower sides at each time point (**P < 0.001). In all panels, average values are shown and error bars are s.e.m. Scale bars, 5 μm (b, d, e, g) and 30 μm (k).
Extended Data Figure 2 | Functional inactivation of ABP1 results in microtubule defects gradually increasing with time of ABP1 inactivation. 

a, b. MAP4–GFP visualization of microtubule orientation in WT and tir1-1 afb1-1 afb2-1 afb3-1 (abbreviated as tir1afb1,2,3) seedlings following 100 nM NAA treatment for 60 min. The proportion of cells with the four categories of microtubule orientation patterns was determined, and Student’s t-test was calculated for the category of transverse microtubule compared with WT treated in the same condition (**P < 0.01).

c–f, MAP4–GFP visualization and quantification of microtubule orientation in roots (c, d) or dark-grown hypocotyls (e, f) of WT, SS12S and SS12K seedlings following different times of ethanol induction as indicated. Student’s t-test was calculated for the transverse microtubules compared with WT exposed for the same time to ethanol vapours as the conditional ABP1 lines (*P < 0.05, **P < 0.001). In all panels, average values are shown and error bars are s.e.m. Scale bars, 5 μm (a, c) and 10 μm (e).
Extended Data Figure 3 | ABP1 is involved in microtubule rearrangement following gravistimulation. a, Rearrangement of microtubules at the lower side compared with the upper side of 90° re-oriented roots of WT, SS12S, SS12K and abp1-5 (all expressing MAP4–GFP). Two different types of microtubule orientation (90 ± 30° or 0–60°/120–180°) were quantified. Student’s t-test was calculated for the category of transverse microtubules compared with each 0° time point and calculated for transverse microtubules in the lower side compared with the upper side at each time point (⁎⁎⁎P < 0.001). b, c, Auxin distribution simulated by DII-VENUS at the lower side compared with the upper side of 90° re-oriented roots of SS12S and SS12K (all in DII-VENUS background; enlarged pictures were visualized in the frames highlighted). Image stacks were taken every 10 minutes, in total for 60 minutes. The ratio of the lower side signal divided by that of the upper side is shown in the chart (c). Student’s t-test was calculated for the signal ratio at each time point of SS12S/K compared with WT (⁎⁎⁎P < 0.001). Signal intensity is represented by the colour code as indicated. Data for SS12S and SS12K (b) are compared with WT (Extended Data Fig. 1i–k). d, The deviated angles of 90° gravistimulated-roots of WT, abp1-5, SS12S and SS12K seedlings were calculated every 30 min, in total for 8 h (Student’s t-test, *P < 0.05, �‡‡P < 0.001). In all panels, average values are shown and error bars are s.e.m. Scale bars, 5 μm (a) and 30 μm (b).
Extended Data Figure 4 | The effect of auxin on fast responsiveness of microtubule dynamics is dependent on ABP1. a, b, Acquisition and quantification of the rate of EB1b movement in roots of untreated or 100 nM NAA-treated (60 min) WT or SS12K (expressing EB1b–GFP) by measuring EB1b–GFP growth events as highlighted by red lines (Student’s t-test, \( P > 0.05 \)). Box plots indicate the 25th centile (bottom boundary), median (middle line), 75th centile (top boundary), the nearest observations within 1.5 times, the interquartile range and outliers. c, EB1b movement was simulated as transverse (blue, 90 ± 30°) or longitudinal (red, 0–60°/120–180°) trajectories before (0°) and after (180°) 100 nM NAA treatment in WT background (colour maps). The blue/red surface ratio is quantified on the chart (\( n = 5 \)). d, Microtubule orientation patterns after 400 µM cordycepin plus NAA co-treatment. Student’s t-test was calculated for the category of transverse microtubules compared with only cordycepin treatment (**\( P < 0.001 \)). e, EB1b trajectories (simulated by time-stack from 10 min videos) were visualized and quantified after DMSO, IAA (1 µM), PEO-IAA (10 µM) and PEO-IAA (10 µM) plus IAA (1 µM) treatments. The left panel shows successive frames of 90° acquisitions following IAA application of pre-treated PEO-IAA WT roots. Student’s t-test was calculated for the category of transverse microtubules compared with DMSO treatment at each time point (**\( P < 0.001 \)). f–i, Projections of EB1b–GFP in SS12K roots (f) and quantification (g) from every 15 s acquisition during 10 min (Supplementary Videos 4 and 6) following DMSO or 100 nM NAA application (\( n = 10 \)). Blue and red strips represent transverse (90 ± 30°) and oblique/longitudinal (0–60°/120–180°) directions, respectively (f). Colour maps show the simulated transverse or longitudinal trajectories of EB1b before (0°) and after (180°) 100 nM NAA treatment in SS12K (h) or SS12S (i) roots. The blue/red surface ratio is quantified on the charts (\( n = 5 \)). The data of SS12S (i) correspond to Fig. 3a, and the data of SS12S and SS12K (f–i) are compared with WT (Fig. 3a and Extended Data Fig. 4c). In all panels except b, average values are shown, error bars are s.e.m. and scale bars are 5 µm.
Extended Data Figure 5 | Overexpressed ABP1-induced effect of auxin on fast responsiveness of microtubule dynamics. a–c, ABP1 and ABP1–GFP transcripts (a) and ABP1 protein level (b, c) were detected in WT and XVE > ABP1-OE line before and after 2 μM oestradiol induction for 12 h or 48 h before RNA or protein extraction. The transcript levels of ABP1 in WT with DMSO treatment were standardized as ‘1’ (a). The 22 kDa native ABP1 band and 49KD ABP1–GFP band were detected and quantified in the right chart. The protein level of native ABP1 or ABP1–GFP in WT was standardized as ‘1’ for each ABP1 and ABP1–GFP, respectively (b, c). Student’s t-test, **P < 0.01. d, Time-lapse observation of microtubule orientation in the roots of XVE > ABP1-OE roots expressing TUA6–RFP, WT and abp1-5 (both expressing MAP4–GFP) upon 100 nM NAA treatment. The percentage of re-oriented microtubules (0–60°/120–180°) was quantified. Re-oriented microtubules in the inducible XVE > ABP1-OE TUA6–RFP roots were calculated compared with none-inducible roots, and abp1-5 MAP4–GFP was compared with MAP4–GFP at each time point (Student’s t-test, *P < 0.05, **P < 0.001). In all panels, average values are shown, error bars are s.e.m and scale bars are 5 μm.
Extended Data Figure 6 | Calcium starvation disrupts microtubule orientation and high calcium increases microtubule depolymerization. Orientation and polymerization statuses of microtubules were visualized following transfer of seedlings to different concentrations of CaCl₂ for 30, 90 or 180 min. Low calcium levels disrupted microtubule organization, leading to a predominantly random pattern; high calcium caused microtubule depolymerization. Student’s t-test was calculated for the category of transverse microtubules compared with seedlings grown and transferred on standard 1/2 Murashige and Skoog medium (with 1.5 mM CaCl₂) (**P < 0.05, ***P < 0.001). In all panels, average values are shown, error bars are s.e.m. and scale bars are 5 μm.
Extended Data Figure 7 | Auxin–ABP1 controls microtubule arrangement through downstream ROP6–RIC1–KTN1 signalling. a, MAP4–GFP visualization of microtubule orientation in the root of WT, rop6-1, ric1-1, SS12S ric1-1 and SS12K ric1-1 following DMSO application for 60 min. Pictures in a correspond to quantifications in Fig. 4a. b, c, Microtubule re-orientation patterns were visualized by MAP4–GFP in the roots of WT and rop6-1/+ following DMSO or 100 nM NAA application for 60 min (Student’s t-test, P > 0.05). d, Transcript level of the scFv12 coding the recombinant antibody responsible for ABP1 knockdown in WT, ric1-1, ktn1, SS12S, SS12K, SS12S ric1-1, SS12K ric1-1, SS12S ktn1 and SS12K ktn1 after 48 h ethanol induction. The transcript level of the scFv12 in SS12S was standardized as ‘1’ (Student’s t-test, P > 0.05). e, Microtubule orientation by MAP4–GFP in dark-grown hypocotyls of WT, SS12K, ktn1 and SS12K ktn1 (with 24 h ethanol induction) following DMSO application for 60 min. Pictures in e correspond to Fig. 4b. In all panels, average values are shown and error bars are s.e.m. Scale bars, 5 μm (a, b) and 10 μm (e).