Supporting Information

Li et al. 10.1073/pnas.1118560109

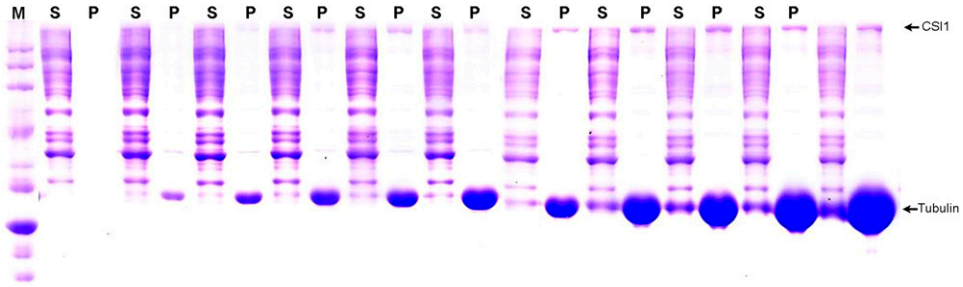


Fig. S1. Microtubule-binding assay of CESA interactive protein 1 (CSI1). For measurement of binding affinity, increasing amounts of taxol-stabilized tubulin were incubated with 0.7 μM purified CSI1 protein for 30 min at room temperature. After centrifugation, 5 μL of each pellet was resolved by SDS/PAGE and visualized by Coomassie blue staining. A representative gel from three technical replicates used for dissociation constant calculation is shown. The positions of CSI1 and tubulin are indicated by arrows. S, supernatant fraction; P, pellet fraction.

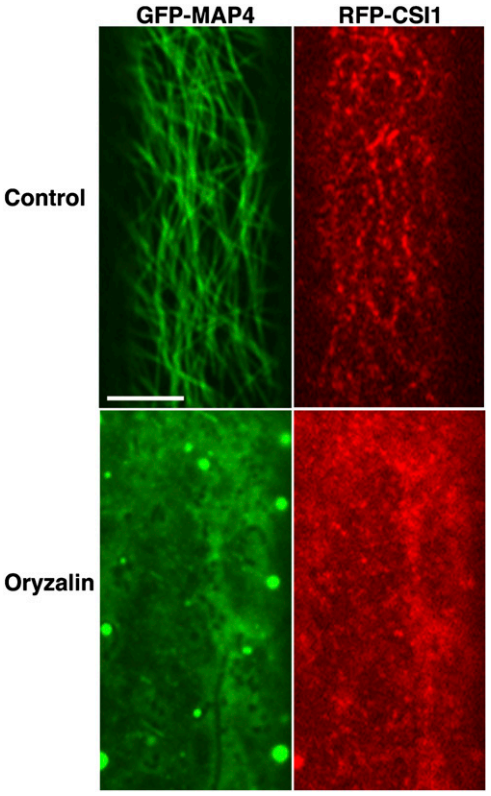


Fig. S2. CSI1 localization is microtubule dependent. Epidermal cells in 3-d-old dark-grown hypocotyls coexpressing GFP-MAP4-MBD and RFP-CSI1 were incubated in Murashige and Skoog liquid solution containing diluted methanol control or 20 μM oryzalin for 7 h. A single optical section of GFP-MAP4-MBD or RFP-CSI1 was acquired. Representative images from 22 cells in nine seedlings upon oryzalin treatment are shown. (Scale bar, 5 μm.)

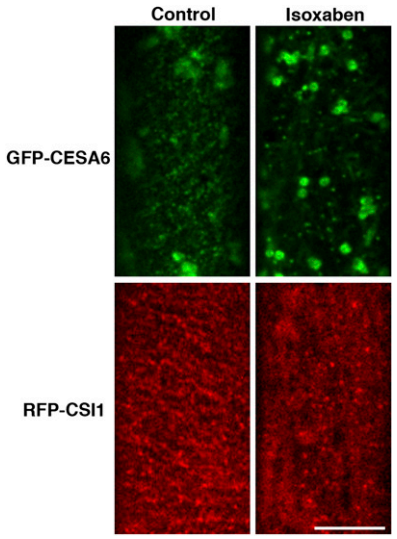


Fig. S3. CSI1 localization requires the integrity of CESA complexes. Epidermal cells in 3-d-old dark-grown hypocotyls expressing GFP-CESA6 or RFP-CSI1 were incubated in Murashige and Skoog liquid solution containing diluted DMSO control or 100 nM isoxaben for 30 min. Representative optical sections of GFP-CESA6 (n = 15 cells) or RFP-CSI1 (n = 21 cells) are shown. (Scale bar, 5 μm.)

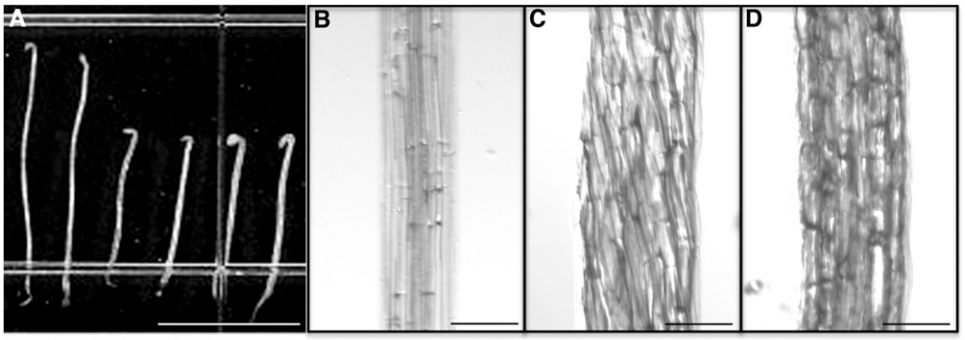


Fig. S4. Oryzalin phenocopies anisotropic growth defect in *csi1* hypocotyls. (*A*) Four-day-old dark-grown hypocotyls. Pairs of seedlings from left to right are the following: Col-0, Col-0 treated with 200 nM oryzalin, and *csi1-3*. (Scale bar, 5 mm.) (*B*–*D*) High magnification of growth morphology of 4-d-old dark-grown wild-type hypocotyls with mock treatment (*B*) and with 300 nM oryzalin (*C*). Anisotropic growth defect of wild type with 300 nM oryzalin was indistinguishable from *csi1-3* (*D*). (Scale bar, 5 μm.)

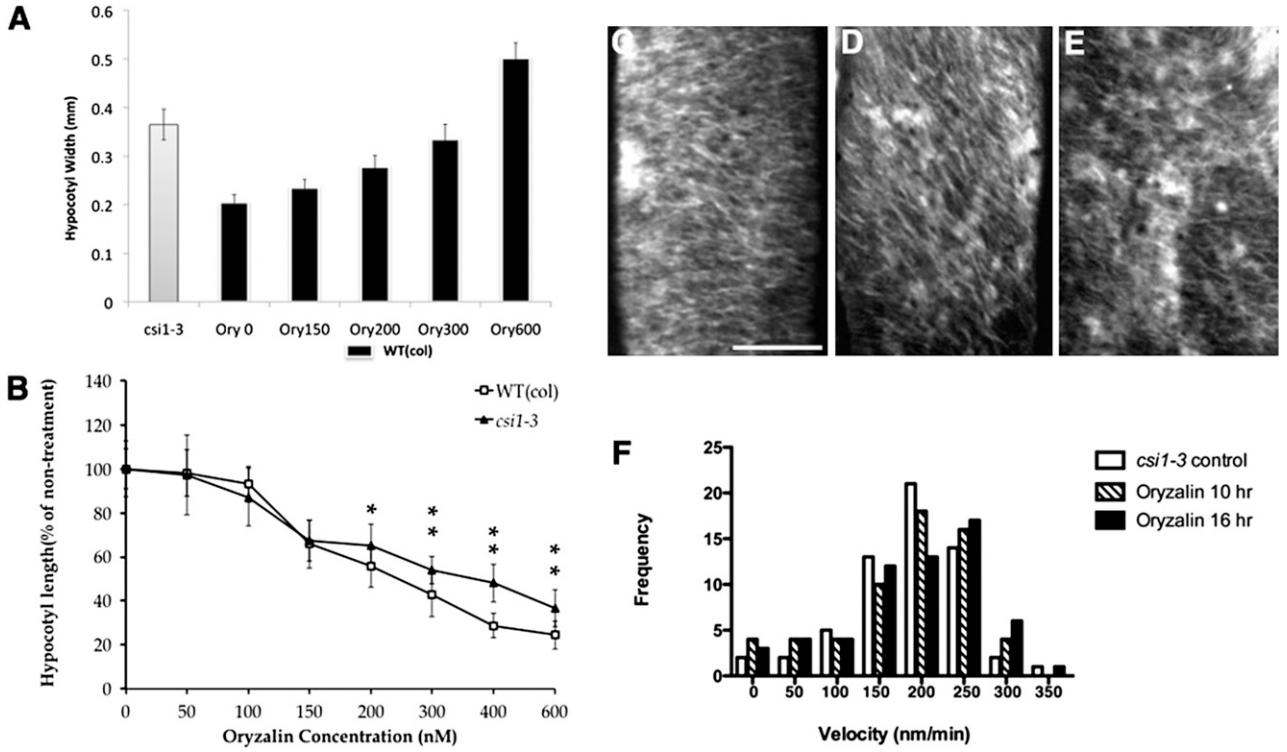


Fig. S5. Quantification of the effect of oryzalin on growth morphology and CESA complexes dynamics in *csi1*. (*A*) Quantification of hypocotyl width in wild type treated with various concentrations of oryzalin and *csi1-3*. (*B*) Dose–response curve of the effect of oryzalin on hypocotyl length. Seedlings were grown on agar for 4 d on Murashige and Skoog plates supplemented with indicated concentrations of oryzalin. $^{**}P < 0.01$; $^{*}P < 0.05$. Error bars indicate SD. (*C*–*E*) Time average of 61 frames (duration 300 s, 5-s interval) showing YFP-CESA6 in *csi1-3* with mock treatment (*C*), with oryzalin treatment for 10 h (*D*), and with oryzalin treatment for 16 h (*E*). (Scale bar, 5 μm.) (*F*) Histogram of YFP-CESA6 particle velocities in mock treatment or oryzalin treatment for indicated time. The average velocity of GFP-CESA6 particles in *csi1-3* (n = 15 cells, 213 ± 70 nm/min) was no different from those treated with 20 μM oryzalin for 10 h (n = 12 cells, 207 ± 87 nm/min) or those treated with 20 μM oryzalin for 16 h (n = 13 cells, 217 ± 89 nm/min).

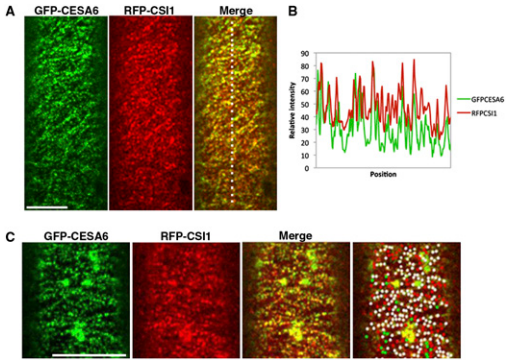


Fig. S6. Colocalization of cellulose synthase (CESA) complexes and CSI1. (*A*) Two-channel imaging of epidermal cells in 3-d-old dark-grown hypocotyls expressing markers for CESA complexes (GFP-CESA6) and CSI1 (RFP-CSI1). A single optical section was acquired. (Scale bar, 5 μm.) (*B*) Plot of a line scan showing a strong correlation between GFP-CESA6 and RFP-CSI1; line is indicated in merge image (*A*). (*C*) Quantification of colocalization of CESA complexes and CSI1. White dots represent colocalized GFP-CESA6 and RFP-CSI1. RFP-CSI1 and GFP-CESA6 that did not colocalize are green and red, respectively. Analysis was performed in five cells from five seedlings (Table 1). (Scale bar, 10 μm.)

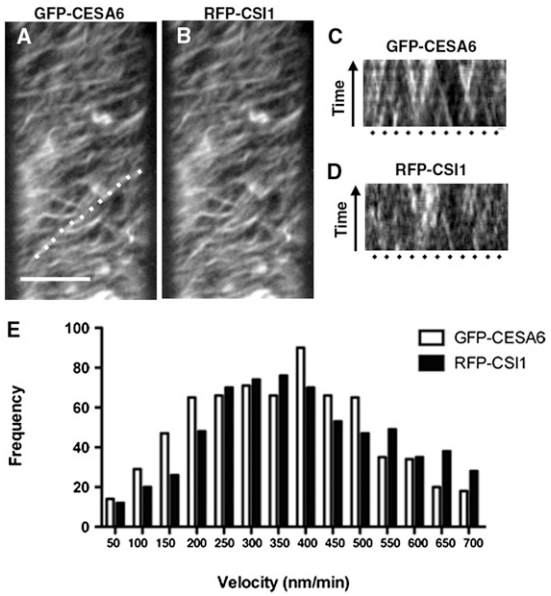


Fig. S7. Velocity measurement of CSI1 and CESA6. Two-channel imaging of epidermal cells expressing both GFP-CESA6 and RFP-CSI1. Time average of 61 frames (duration 300 s, 5-s interval) showing a similar linear track of GFP-CESA6 (*A*) and RFP-CSI1 (*B*). (*C* and *D*) Kymograph of region highlighted in *A*. (*E*) Histogram of GFP-CESA6 and RFP-CSI1 particle velocities. A representative image from six cells used for analysis is shown. The mean velocity was 337 ± 157 nm/min for RFPCSI1 (n = 686 particles) and 361 ± 163 nm/min for GFP-CESA6 (n = 646 particles). (Scale bar, 5 μm.)

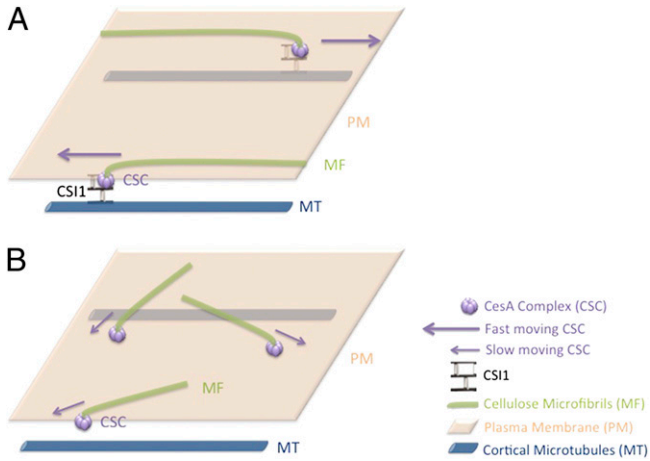
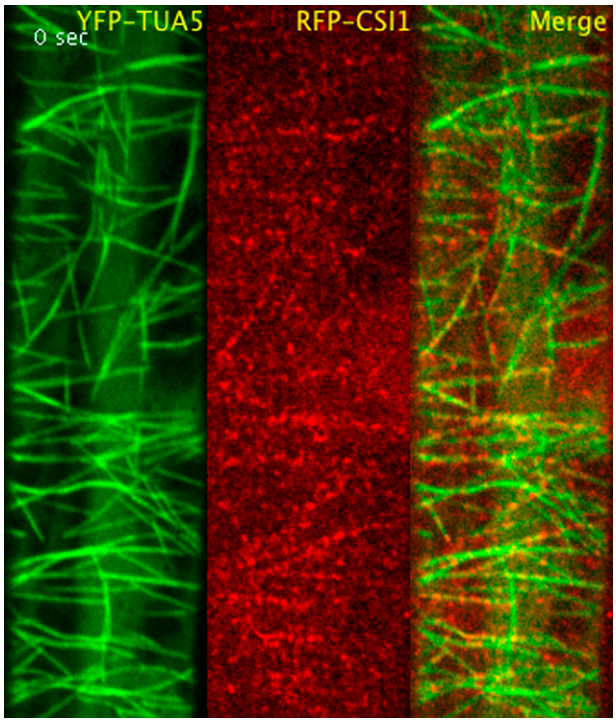
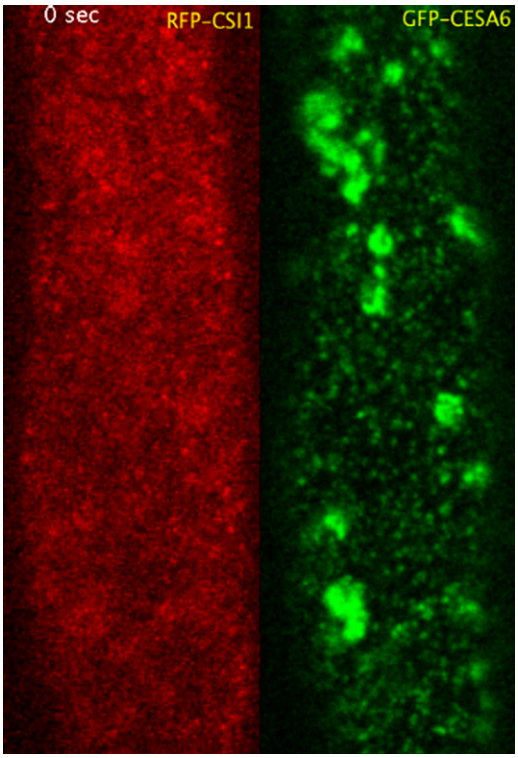


Fig. S8. Potential mechanisms for microtubule guidance of synthesis of cellulose microfibrils. We propose that CSI1 is the linker protein that bridges between cortical microtubules and CESA complexes. (*A*) In wild-type cells, CESA complexes are positioned along the cortical microtubules through CSI1. The orientation of cellulose microfibrils mirrors that of underlying cortical microtubules. (*B*) In cells where CSI1 is lost, CESA complexes are misaligned with cortical microtubules. Without the molecular track, one CESA complex may bump into another, resulting in the interference of efficient cellulose synthesis, which is represented by slow-moving CESA complexes. This phenomenon can also be phenocopied by removing cortical microtubules by treatment with oryzalin.



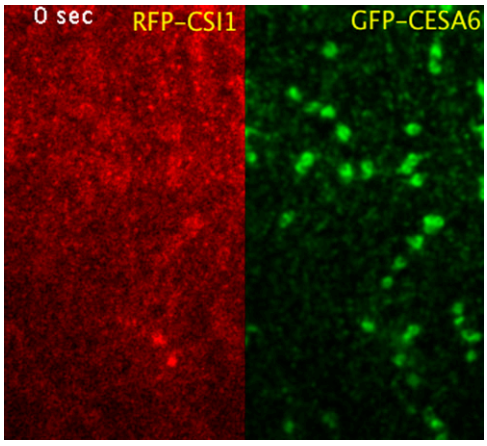
Movie S1. CSI1 particles travel along cortical microtubules. Epidermal cells in 3-d-old dark-grown hypocotyls coexpressing RFP-CSI1 and YFP-TUA5 were imaged in the plane of plasma membrane. Time series are 5 min long at 5-s intervals. Frame rate = 7 fps.

Movie S1



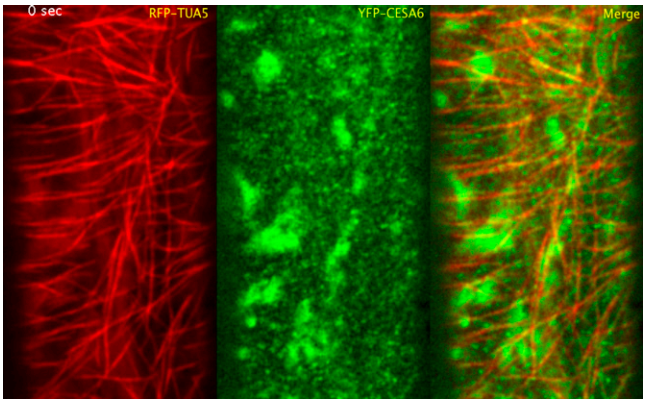
Movie S2. Two-channel imaging of epidermal cells in 3-d-old dark-grown hypocotyls coexpressing RFP-CSI1 and GFP-CESA6. Two-day-old dark-grown hypocotyls were incubated in Murashige and Skoog liquid solution containing 20 μM oryzalin for 10 h before imaging. Time series are 5 min long at 5-s intervals. Frame rate = 7 fps.

Movie S2



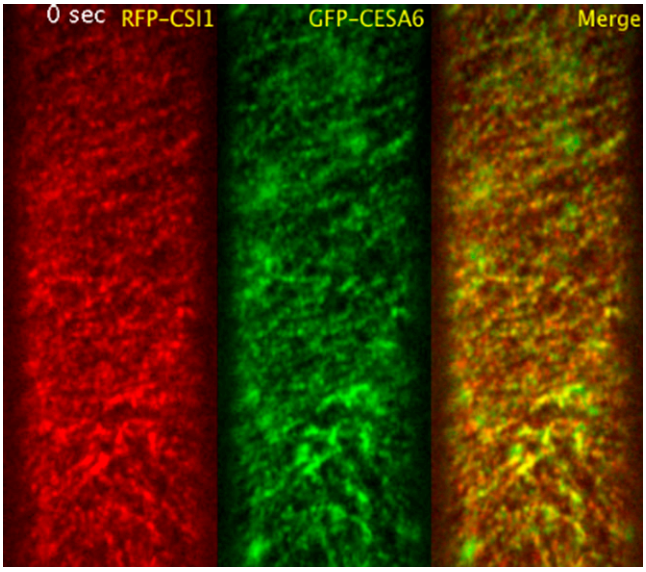
Movie S3. Two-channel imaging of epidermal cells in 3-d-old dark-grown hypocotyls coexpressing RFP-CSI1 and GFP-CESA6. Two-day-old dark-grown hypocotyls were incubated in Murashige and Skoog liquid solution containing 20 μM oryzalin for 16 h before imaging. Time series are 5 min long at 5-s intervals. Frame rate = 7 fps.

Movie S3



Movie S4. Two-channel imaging of epidermal cells in 3-d-old dark-grown hypocotyls coexpressing RFP-TUA5 and YFP-CESA6 in *csi1-3*. Time series are 2 min long at 5-s intervals. Frame rate = 7 fps.

Movie S4



Movie S5. Dynamic association of CESA and CSI1. Epidermal cells in 3-d-old dark-grown hypocotyls coexpressing RFP-CSI1 and GFP-CESA6 were imaged in the plane of plasma membrane. Majority of CESA and CSI1 particles travel simultaneously along a linear track. Time series are 5 min long at 5-s intervals. Frame rate = 7 fps.

Movie S5