Supporting Information

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SI Materials and Methods

Plant Growth Conditions and Mutant Screen. *Arabidopsis thaliana* seeds (Columbia) were sown on one-third-strength Murashige and Skoog culture medium containing 1% (wt/vol) sucrose with 0.8% (wt/vol) agar and incubated for 2 d at 4 °C. After incubation, they were grown under white light at ≈100 μmol m^{-2} s^{-1} under a light (16 h)/dark (8 h) cycle at 23 °C in a growth chamber. Plants were grown for 2 wk for the screen and mapping of mutant plants and for 3 wk for analysis of chloroplast movements and cytoplasmic streaming. For protoplast isolation, plants were grown in soil for 3 wk. γ-ray and fast neutron-mutagenized seeds (Lehle Seeds) were used to screen mutants. The techniques for the mutant screen using the white band assay (WBA) and genetic mapping were previously reported (1–3). The transferred DNA (T-DNA) tagged lines, SALK and GABI-Kat (4), were provided by the Arabidopsis Biological Resource Center and the European Arabidopsis Stock Center, respectively.

cDNA Cloning and RT-PCR. Full-length cDNAs were isolated by the 5′ and 3′ rapid amplification of cDNA ends method using the 5′- and 3′-RACE System kits, according to the manufacturer's instructions (Invitrogen). Total RNA was extracted from leaves, stems, and roots of 7-wk-old plants for RT-PCR. The first-strand cDNAs were synthesized from total RNA using oligo-dT primers. The amounts of cDNA were normalized using the *β-tubulin 2* (*TUB*) and *ubiquitin-conjugating enzyme* (*UBC*) genes as internal controls. The DNA fragments for *WEB1*, *PMI2*, *TUB*, and *UBC* were amplified by PCR with the following primers: *WEB1* [5′-AAGTCAATCCTGAGTTGATTGATTCCACCA-3′ and 5′-TTCCAGTGCTCACCGGTGATCCTGA-3′], *PMI2* [5′-GGAATTTAGATGGAACTGTATCAG-3′ and 5′-CCTTAGCAGACTCAGCAACTC-3′], *TUB* [5′-CTCTGACCTCCGAAAGCTTGC-3′ and 5′-TCACCTTCTTCATCCGCAGTT-3′], and *UBC* [5′-TCAAATGGACCGCTCTTATCAAAGG-3′ and 5′-TCCAACCAGGACTCCAAGCATTC-3′].

Analyses of Chloroplast Movement. Detached rosette leaves from seedlings were used for the WBA. The leaves were placed on a 0.8% agar plate with their adaxial sides up and enclosed with transparent film. The film-enclosed leaves were covered with a non–light-transmitting black plate with 1-mm-wide open slits and irradiated with continuous white light (500 and 1,200 μmol m^{-2} s^{-1}) in a growth chamber with a 3.6-kW xenon lamp (Ushio) at 22 °C and 50% humidity. The individual detached leaves were observed after the appropriate irradiation time. We developed an evaluation method for the WBA. Because plant absorbs blue and red, not green, lights for photosynthesis and photoresponses, the blue and red lights are hard to be transmitted through leaf. We took advantage of the phenomenon for developing the evaluation method. To remove the information of green channel image, we first split the RGB image into three independent channel images. Under our experimental condition, red light was likely more transmitted through the leaf, compared with blue light. This effect might depend on spectral characteristics of the light source. Nevertheless, because the white band in the blue channel image was visibly clearer than that in the red channel image, we decided to use only the blue channel image to develop a simple evaluation method for WBA. Blue channel images were isolated from the RGB photographs using Java-based ImageJ software (http://rsb.info.nih.gov/ij/). The midpoints (pixel 20) of the long sides of the region of interest (ROI; 10 × 40 pixels) were assigned to the midpoint of the width of the irradiated area, such that the lines of the long sides were perpendicular to the irradiated area (Fig. 3*B* in main text). A pixel profile of the ROI was then obtained using the ImageJ command function. The chloroplast velocity was determined by blue light microbeam irradiation with a custom-made system that has been described previously (5). After recording the movement time (t) and distance (d) that a chloroplast moved, the velocity (v) of chloroplast movement was calculated with the equation v = d/t. For analysis of leaf transmittance changes, detached leaves (from 16- to 19-d-old seedlings) were placed on 1% gellan gum medium in a 96-well plate, and the red light (650 nm) transmittance was automatically recorded every 2 min using a microplate reader (VersaMax; Molecular Devices). Blue light was provided from a blue light-emitting diode illuminator (LED-mB; EYELA).

Protein Analyses. The N-terminal region (1–134 aa) of WEB1 was subcloned into the pGEX-2T vector, which contains the *GST* gene (Amersham Pharmacia). The GST fusion protein was expressed in the *Escherichia coli* BL21 strain with 1 mM isopropyl-b-D-thiogalactoside (IPTG) and purified using glutathione-Sepharose 4B beads (Amersham Pharmacia). The WEB1 fragment was eluted by cleavage of the GST-WEB1 fusion protein with the Thrombin protease (Amersham Pharmacia). Polyclonal antisera against the fusion protein raised in rabbit were used as an anti-WEB1 antibody for immunoblot analysis. Protein extracts were prepared from the rosette leaves of 4-wk-old plants grown in soil. The leaves were ground in extraction buffer containing 50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 2 mM EDTA, 1 mM $MgCl_2$, 10% glycerol, and 1 tablet (per 25 mL) of complete proteinase inhibitor mixture (Roche) and homogenized with a glass homogenizer. After centrifugation at 11,000 × *g* for 15 min at 4 °C twice to remove insoluble debris, crude extracts were further fractionated by ultracentrifugation at 100,000 × *g* for 1 h at 4 °C. The resulting supernatant and pellet were used as the soluble (S) and microsomal (P) protein fractions, respectively. For immunoblot analysis, 50 μg of protein sample was separated on 7.5% SDS/PAGE gels. The separated proteins were electronically transferred onto nitrocellulose membrane, and WEB1, phot1, and phot2 were detected with anti-WEB1, anti-phot1 (6), and anti-phot2 (7) polyclonal antibodies, respectively. Alkaline phosphatase-conjugated goat anti-rabbit IgG (Promega) was used as a secondary antibody. For size exclusion chromatography, the soluble fractions were prepared from 4-wk-old rosette leaves, as described above. Approximately 2 mg of the protein sample in a 0.5-mL injection volume was separated in a Superdex 200 10/300 column connected to an ÄKTAexplorer 10S (GE Healthcare). The fractionated protein extracts were collected in 0.5-mL fractions and precipitated with 0.5 mL of cold trichloroacetic acid (TCA)–acetone solution (10% TCA in cold acetone). The protein pellets were rinsed twice with cold acetone and dissolved and denatured in 50 μL of 1× SDS/PAGE sample buffer, and half of the protein sample was subsequently used for immunoblot analysis. In vitro transcription, translation, and expression were carried out as previously described (8) and according to the manufacturer's instructions (Promega). The cDNA for WEB1 was subcloned into the pET22(b)+ vector, and the WEB1-6xHis protein was expressed in the *E. coli* BL21 (DE3) strain. The cells were boiled and centrifuged at 11,000 × *g* for 10 min to remove the insoluble fraction. The WEB1-6xHis fusion protein was detected with a mouse anti-His antibody and alkaline phosphatase-conjugated anti-mouse antibody (Promega).

Analyses with Fluorescent Proteins. Plasmids containing the genes for GFP-WEB1 and GFP-PMI2 were constructed by subcloning the cDNA fragments into the *Cauliflower Mosaic Virus* (*CaMV*) *35S-sGFP(S65T)-Nos* vector, which harbors a synthetic gene for improved GFP, sGFP(S65T) (9). cDNA fragments of *WEB1* and *PMI2* were subcloned into the BsrGI/NotI site. For the *CaMV35S-CFP-WEB1-Nos*, *CaMV35S-YFP-PMI2-Nos*, and *CaMV35S-DsRed-monomer-Nos* plasmids, *CFP*, *YFP*, and *DsRed-monomer* (Clontech), respectively, replaced the *GFP*. For YN-WEB1 and YN-PMI2, *WEB1* and *PMI2* were subcloned into the pSY736 vector, and for YC-WEB1 and YC-PMI2, the cDNAs were subcloned into the pSY735 vector (10).

For transient expression in *Arabidopsis* protoplasts, protoplasts were isolated from *Arabidopsis* rosette leaves, and the appropriate vectors were introduced into the cells via PEG-mediated transformation (9). After incubation at 25 °C for 24 h in the dark, the cells were observed under a confocal microscope (SP5; Leica). For transient expression in the onion epidermal cell layer, the appropriate vectors were introduced into onion epidermal cells using the particle bombardment method. After incubation at 25 °C for 12 h in the dark, the cells were observed under a microscope equipped with a fluorescence module (Imager; Carl Zeiss) (11) and a confocal microscope (SP2; Leica).

Yeast Two-Hybrid Analysis. The binding domain (BD) plasmids containing the genes for BD-WEB1 and BD-PMI2 were constructed by subcloning the *WEB1* and *PMI2* cDNAs, respectively, into the pGBKT7 vector (Clontech). The activation domain (AD) plasmids containing the genes for AD-WEB1, AD-PMI2, and AD-JAC1 were constructed by subcloning the *WEB1*, *PMI2*, and *JAC* cDNAs, respectively, into the pGADT7 vector (Clontech). The BD and AD plasmids were transformed into the yeast strains AH109 and Y187, respectively (Clontech). Transformants expressing the BD fusions were then mated with transformants expressing the AD fusions. The resulting mated transformants were used in experiments for complementation of growth on SD agar supplemented with a mixture of the appropriate amino acids without Trp, Leu, His, and Ade.

Cytoplasmic Streaming. The method used to measure red light-induced cytoplasmic streaming has previously been reported (12). A detached leaf was transferred into a cuvettete composed of two round coverslips supported by a ring-shaped silicon–rubber spacer (12) and irradiated with red light under a microscope (5). The red light was supplied by a halogen lamp (Focusline 12V-100W HAL; Philips Lighting) equipped with a red plastic filter (Shinkolite A no. 102; Mitsubishi Rayon).

Observation of cp-actin Filaments. Transgenic *Arabidopsis* plants expressing GFP-tagged talin (the F-actin binding domain of mouse talin), which has been described previously (13), were crossed with the *web1*, *pmi2*, and *web1pmi2* mutant lines. F3 homogenous lines were selected and used for cp-actin observation. Rosette leaves of 4-wk-old plants grown in soil under a light (16 h)/dark (8 h) cycle at 23 °C were detached and evacuated gently in a syringe filled with deionized water. The leaves were set into a cuvettete composed of two round coverslips supported by a ring-shaped silicon–rubber spacer (12). cp-actin filaments were visualized by observation of GFP-tagged talin using a confocal microscope (SP5; Leica). A multi argon laser was used to excite GFP (488 nm) and induce the chloroplast avoidance response (458 nm). Four images in 0.67-μm steps were captured with a lens (63×/1.20W) at a resolution of 256 × 256 using a 4× digital zoom for 30 cycles, and the stimulating laser (458 nm, 2.8 μW) scanned the rectangular ROI (15 μm × 50 μm) for 24 s between intervals. The images were enlarged using the LAS imaging software (Leica).

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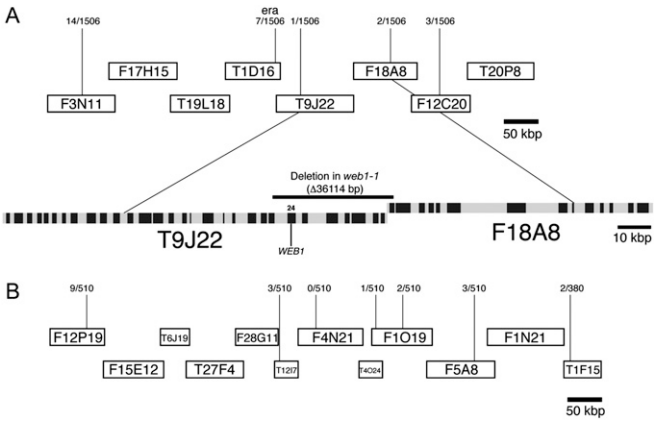


Fig. S1. Map-based cloning. (*A*) Positional cloning of the *WEB1* gene. The *web1* mutation was mapped near the cleaved amplified polymorphic sequence marker on the lower arm of chromosome 2. Analysis of 1,506 chromosomes derived from the segregating F2 mutant plants from crosses between *web1-1* in the Columbia (Col-0) background and Landsberg *erecta* (L*er*) narrowed the position of *WEB1* to a region in two bacterial artificial chromosomes (BACs), T9J22 and F18A8. The number of recombinants is indicated. Genomic PCR analysis revealed that *web1-1* had a large deletion of genomic DNA containing eight genes. Phenotypic analysis of T-DNA insertion lines and sequencing analysis of *web1-2* indicated that *WEB1* is *At2g26570*. (*B*) Positional cloning of the *WEB2* gene. The *web2* mutation was mapped between the simple sequence length polymorphism markers F12P19 and T1F15 on the lower arm of chromosome 1. Analysis of 510 chromosomes derived from the segregating F2 mutant plants from crosses between the *web2-1* Col-0 background line and L*er* narrowed the position of *WEB1* to a region in two BACs, F4N21 and T4O24. The number of recombinants is indicated. Genomic PCR and RT-PCR analyses revealed that *web2-1* had a large deletion of genomic DNA containing 11 genes, although the precise position of the deletion remains to be determined. Phenotypic analysis of a T-DNA insertion line (*pmi2-2*) indicated that WEB2 is *At1g66840,* which is the *PMI2* gene.

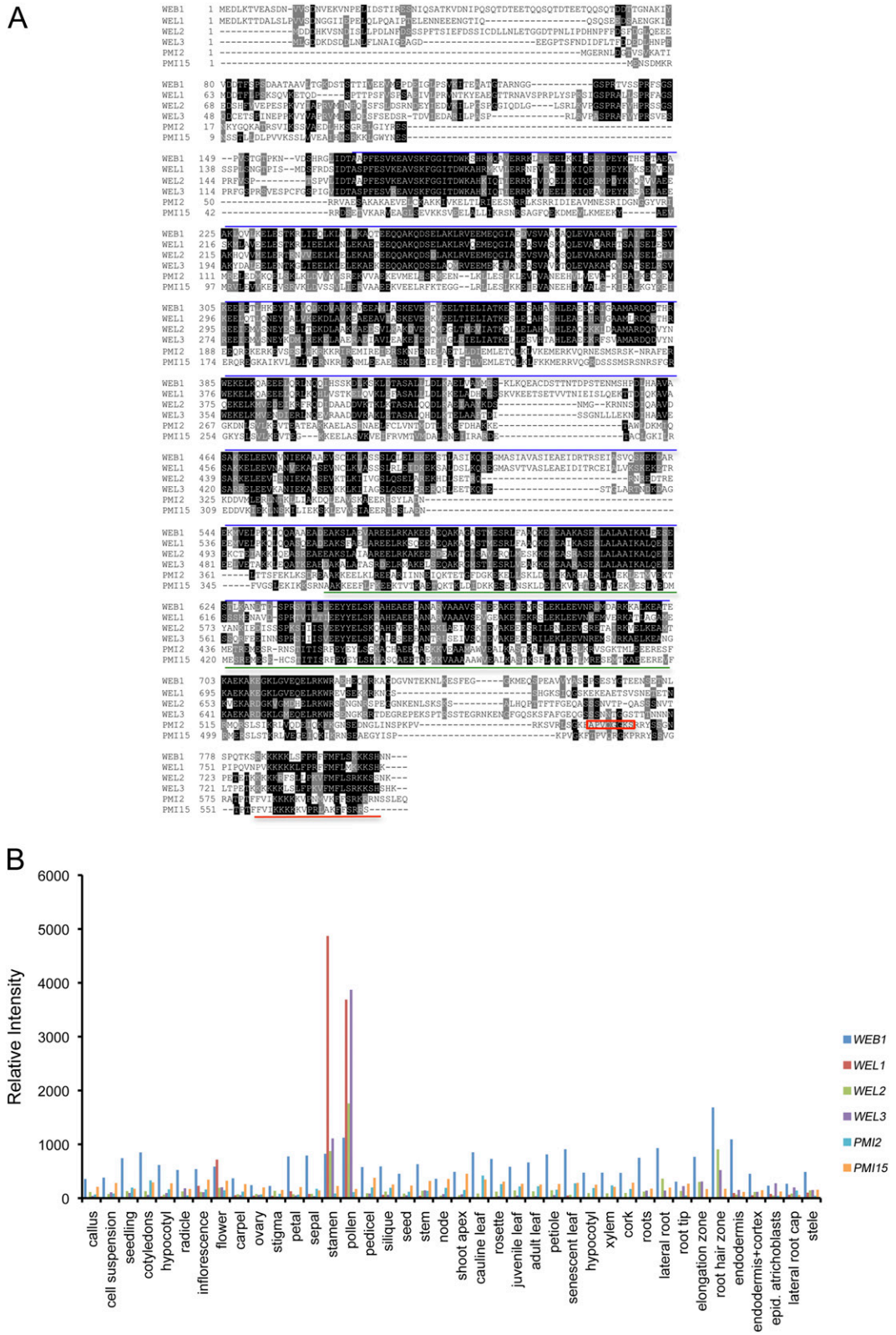


Fig. S2. *WEB1* and *PMI2* family genes and their gene expression patterns. (*A*) Comparison of the WEB1, PMI2, WEL1, WEL2, WEL3, and PMI15 sequences. Black and gray boxes indicate identical and similar amino acids, respectively. The blue upper line indicates the DUF827 region. The green underline indicates the region used for construction of the phylogenetic tree in Fig. 1*E* (main text). The red underline indicates a nuclear localization signal-like motif. The red box indicates the putative ATP/GTP-binding motif A (P-loop) of PMI2. (*B*) Tissue-specific gene expression of *WEB1*, *PMI2*, *WEL1*, *WEL2*, *WEL3*, and *PMI15*. The data were obtained from the Genevestigator public microarray database (https://www.genevestigator.com/).

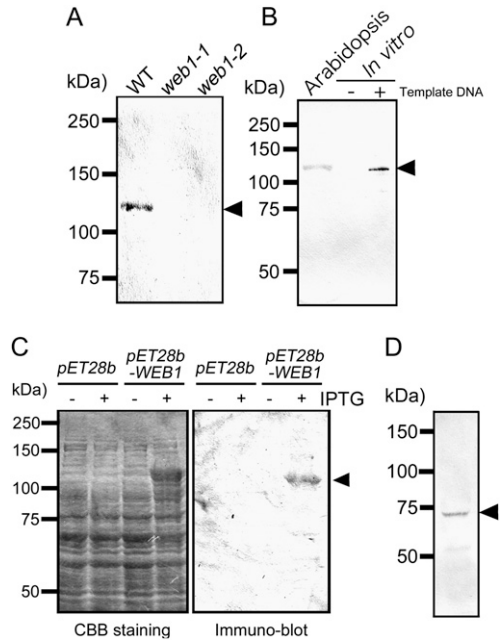


Fig. S3. Molecular masses of the WEB1 and PMI2 proteins. (*A*) Molecular mass of the WEB1 protein in *Arabidopsis*. Crude extracts from *Arabidopsis* rosette leaves (WT, *web1-1*, and *web1-2*) were directly used for immunoblot analysis. After fractionation by SDS/PAGE, proteins were electrically transferred to a nitrocellulose membrane, and WEB1 was detected with an anti-WEB1 polyclonal antibody and an alkaline phosphatase-conjugated anti-rabbit secondary antibody (Promega). Arrowhead indicates the position of the WEB1 protein. (*B*) Expression of the WEB1 protein in the in vitro transcription/translation expression system. The in vitro transcription/translation expression system was used according to the manufacturer's instructions (Promega). (*C*) Expression of WEB1-6xHis protein in *E. coli*. WEB1-6xHis was induced with 1 mM IPTG and detected with a mouse anti-His antibody and an alkaline phosphatase-conjugated anti-mouse secondary antibody (Promega). (*D*) Expression of PMI2-6xHis protein in the in vitro transcription/translation expression system.

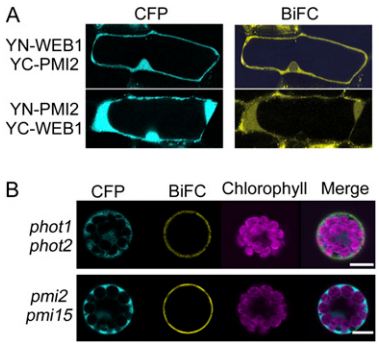


Fig. S4. Bimolecular fluorescence complementation (BiFC) assay. (*A*) Visualization of the WEB1-PMI2 interaction in onion epidermal cells. Onion epidermal cell layers were bombarded with gold particles coated with the appropriate BiFC constructs. The cells were observed under a confocal microscope (SP2; Leica). CFP was used as a cytosolic and nuclear localization control. (*B*) Visualization of WEB1 homodimerization in *phot1phot2* and *pmi2-3pmi15-1*. *Arabidopsis* protoplast cells were isolated from *phot1phot2* and *pmi2-3pmi15-1* plants, and YN-WEB1 and YC-WEB1 were coexpressed in the protoplasts. After a 24-h incubation at 25 °C in the dark, the cells were observed under a confocal microscope (SP5; Leica). (Scale bars, 10 μm.)

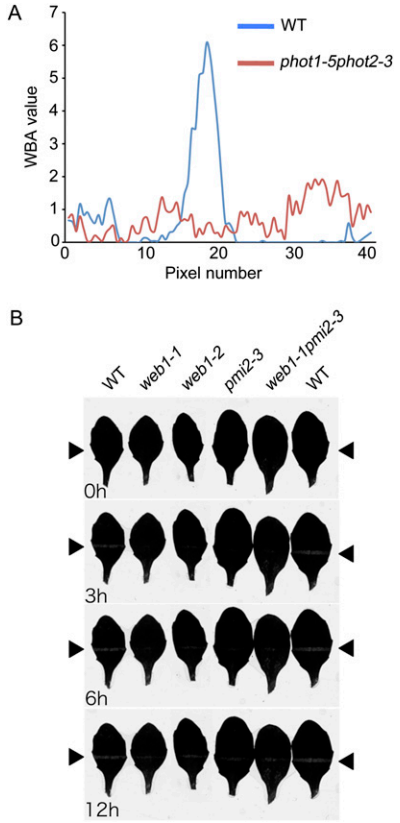


Fig. S5. Evaluation method for WBA and time course experiment. (*A*) Preliminary test. Detached rosette leaves of WT and *phot1-5phot2-3* mutant were aligned on a 0.8% agar plate, covered with a non–light-transmitting black plate with a 1-mm-wide open slit, and irradiated with continuous strong white light (≈800 μmol m^{-2} s^{-1}) for 30 min. Vertical and horizontal axes show the WBA values and pixel numbers of the long side of the ROI, respectively. (*B*) Time course experiment. Detached rosette leaves of WT and mutants were irradiated with a continuous strong white light (500 μmol m^{-2} s^{-1}) for 0, 3, 6, and 12 h in a growth chamber. Arrowheads indicate the irradiated position.

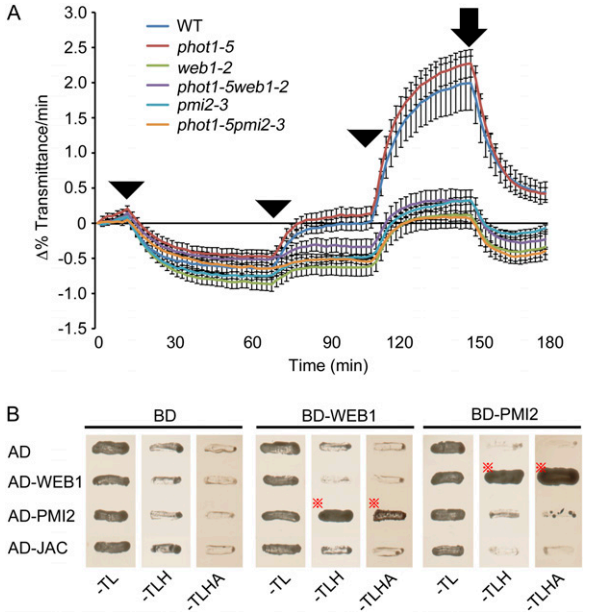


Fig. S6. No apparent genetic interaction with *PHOT1* and no physical interaction with JAC1 in Y2H assay. (*A*) No apparent genetic interactions of *WEB1* and *PMI2* with *PHOT1*. The changes in leaf transmittance caused by chloroplast photorelocation movement were measured in the WT plants and *phot1-5*, *web1-2*, *phot1-5web1-2*, *pmi2-3*, and *phot1-5pmi2-3* mutants. The graph shows data from a representative experiment using eight leaves of each indicated genotype. After 10 min in darkness, leaves were irradiated with blue light at 3, 20, and 50 μmol m^{-2} s^{-1} sequentially at 10, 70, and 110 min, respectively, as indicated by the arrowheads. The blue light was turned off at 150 min (arrow). (*B*) No detection of possible physical interactions of WEB1 and PMI2 with JAC1 in Y2H assay. The Gal4 DNA binding domain (BD) and activation domain (AD) fusion proteins were coexpressed in diploid yeast cells that were constructed via mating. The resulting transformants were used in experiments for complementation of growth on SD agar supplemented with a mixture of the appropriate amino acids without Trp (T), Leu (L), His (H), and Ade (A). Interaction between WEB1 and PMI2 was used as a positive control interaction. Asterisks indicate positive interaction clones.

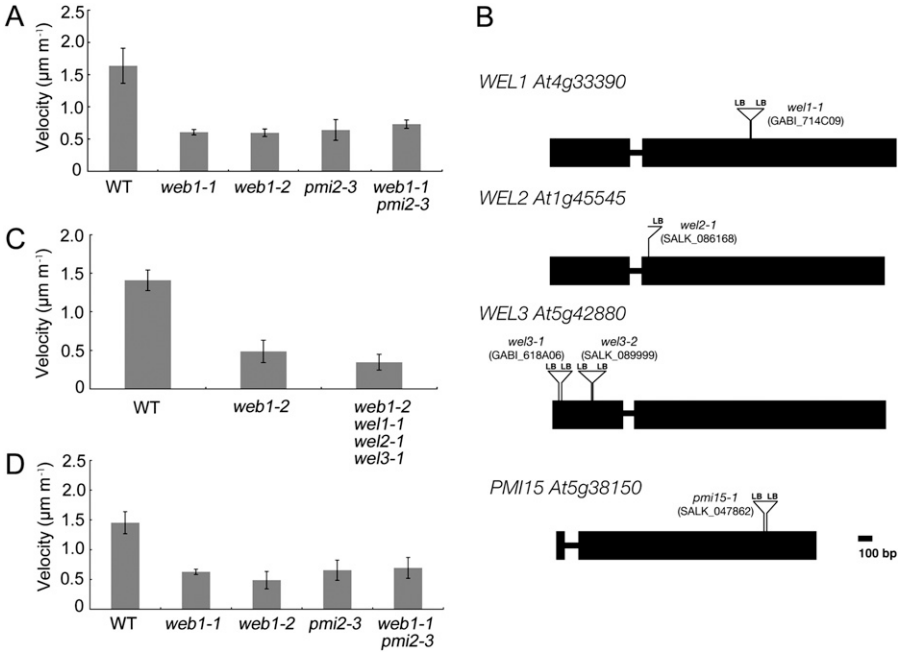


Fig. S7. Direct measurement of chloroplast velocity and *WEB1*- and *PMI2*-like genes. (*A*) Chloroplast velocity in the avoidance response. The blue light microbeam irradiation method was used to calculate chloroplast velocity. To induce the avoidance response, 30 μmol m^{-2} s^{-1} of blue light was irradiated on the mesophyll cells. After recording the time (t) and distance (d) of chloroplast movement, the velocity (v) of chloroplast movement was calculated with the equation v = d/t. (*B*) Schematic illustrations show the gene structures of *WEL1*, *WEL2*, *WEL3*, and *PMI15*. Rectangles and intervening bars indicate exons and introns, respectively. T-DNA insertion sites are also indicated. LB, left border of the T-DNA. The SALK and GABI-Kat mutant lines were obtained from the Arabidopsis Biological Resource Center and the European Arabidopsis Stock Center, respectively. (*C*) Velocity of chloroplasts in the avoidance response in a quadruple mutant plant. Strong blue light (30 μmol m^{-2} s^{-1}) was irradiated on the mesophyll cells of WT, *web1*, and *web1wel1wel2wel3* quadruple-mutant plants to induce the avoidance response. (*D*) Chloroplast velocity in the accumulation response. The blue light microbeam irradiation method was used to calculate chloroplast velocity. To induce the accumulation response, 10 μmol m^{-2} s^{-1} of blue light was shone on the mesophyll cells.

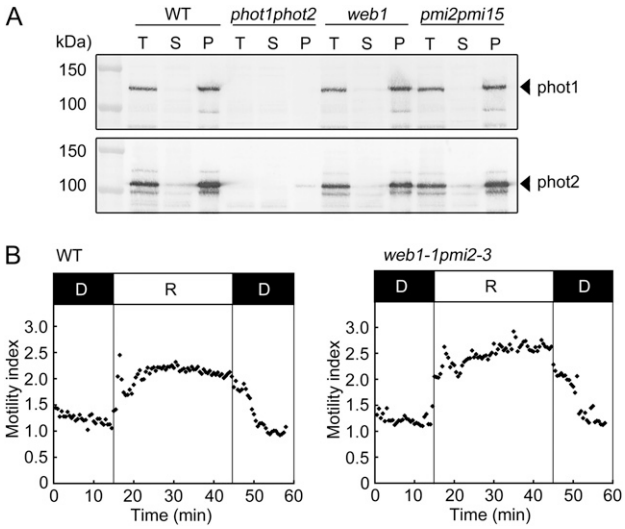
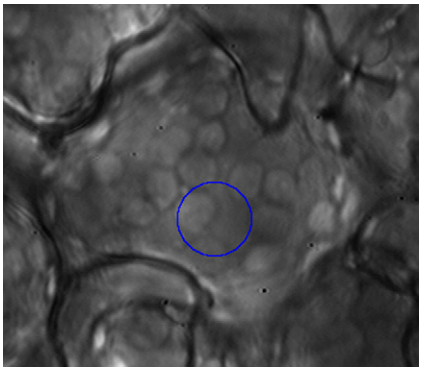
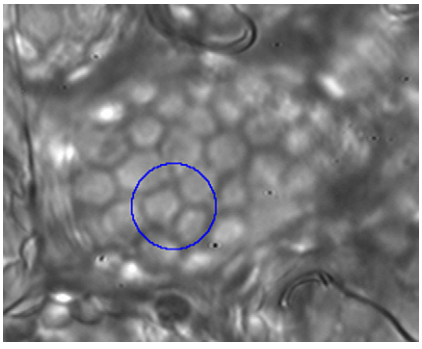


Fig. S8. Phototropin protein accumulation and cytoplasmic streaming. (*A*) Phototropin protein accumulation. Proteins were extracted from WT, *phot1phot2*, *web1-1*, and *pmi2-3pmi15-1* plants and fractionated. The protein fractions were subjected to SDS/PAGE analysis (7.5% gels), and phot1 and phot2 were detected by immunoblot analysis with anti-phot1 and anti-phot2 polyclonal antibodies, respectively. T, total protein extract; S, soluble fraction; P, pelleted microsomal fraction. (*B*) Red light-induced cytoplasmic streaming. Cytoplasmic streaming was induced by red light and observed under a microscope. The calculation procedure used was described in a previous study (12). D, dark conditions (infra-red); R, red light conditions.



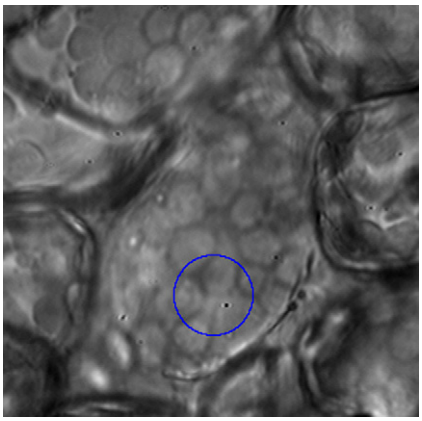
Movie S1. Chloroplast avoidance movement in WT. A mesophyll cell was irradiated with strong blue light (30 μmol m^{-2} s^{-1}). Images were acquired at 1-min intervals for 30 min. Circle indicates the irradiated area.

Movie S1



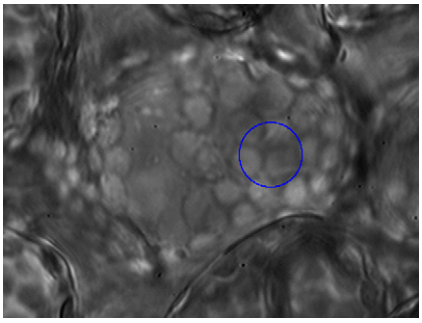
Movie S2. Chloroplast avoidance movement in *web1-1*. A mesophyll cell was irradiated with strong blue light (30 μmol m^{-2} s^{-1}). Images were acquired at 1-min intervals for 30 min. Circle indicates the irradiated area.

Movie S2



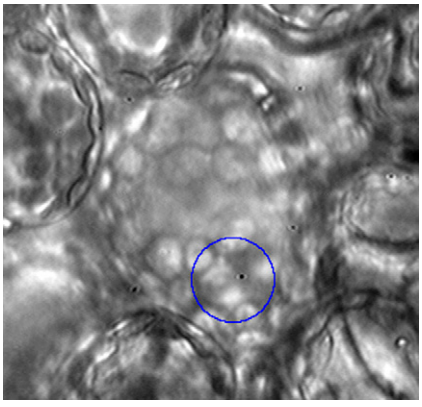
Movie S3. Chloroplast avoidance movement in *web1-2*. A mesophyll cell was irradiated with strong blue light (30 μmol m^{-2} s^{-1}). Images were acquired at 1-min intervals for 30 min. Circle indicates the irradiated area.

Movie S3



Movie S4. Chloroplast avoidance movement in *web2-1/pmi2-3*. A mesophyll cell was irradiated with strong blue light (30 μmol m^{-2} s^{-1}). Images were acquired at 1-min intervals for 30 min. Circle indicates the irradiated area.

Movie S4



Movie S5. Chloroplast avoidance movement in *web1-1pmi2-3*. A mesophyll cell was irradiated with strong blue light (30 μmol m^{-2} s^{-1}). Images were acquired at 1-min intervals for 30 min. Circle indicates the irradiated area.

Movie S5