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Report

THRUMIN1 Is a Light-Regulated Actin-Bundling Protein Involved in Chloroplast Motility

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Summary

Chloroplast movement in response to changing light conditions optimizes photosynthetic light absorption [1]. This repositioning is stimulated by blue light perceived via the phototropin photoreceptors [2–4] and is transduced to the actin cytoskeleton [5]. Some actin-based motility systems use filament reorganizations rather than myosin-based translocations [6]. Recent research favors the hypothesis that chloroplast movement is driven by actin reorganization at the plasma membrane [7, 8], but no proteins affecting chloroplast movements have been shown to associate with both the plasma membrane and actin filaments in vivo. Here we identified THRUMIN1 as a critical link between phototropin photoreceptor activity at the plasma membrane and actin-dependent chloroplast movements. THRUMIN1 bundles filamentous actin in vitro, and it localizes to the plasma membrane and displays light- and phototropin-dependent localization to microfilaments in vivo. These results suggest that phototropin-induced actin bundling via THRUMIN1 is important for chloroplast movement. A mammalian homolog of THRUMIN1, GRXCR1, has been implicated in auditory responses and hair cell stereocilla development as a regulator of actin architecture [9, 10]. Studies of THRUMIN1 will help elucidate the function of this family of eukaryotic proteins.

Results and Discussion

To better understand light-mediated chloroplast movement, we performed a photometric screen for *plastid movement impaired* mutants in *Arabidopsis thaliana* [11]. The *thrumin1-1* mutant was isolated on the basis of slower-than-normal changes in red-light leaf transmittance in response to low and high blue-light treatments (Figure 1A). Chloroplast movement defects of *thrumin1-1* homozygous mutant plants were also observed during blue-light microbeam experiments (see Movie S1 available online). Kinetic analysis of chloroplast movement during these blue-light microbeam assays indicates that the average speed and straightness of chloroplast movements were reduced in *thrumin1-1* mutants compared to wild-type plants (Figures S1A and S1B).

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By recombinant mapping and candidate gene sequencing, two nonsynonymous mutations (*thrumin1-1*^{G2E} and *thrumin1-1*^{E330K}) were identified in the At1g64500 open reading frame in the *thrumin1-1* mutant (Figure 1C). Two independently derived T-DNA insertion mutants (Figure 1A), *thrumin1-2* and *thrumin1-3*, also displayed chloroplast movement defects identical to *thrumin1-1* (Figure 1A), confirming that THRUMIN1 is involved in chloroplast movement. Furthermore, the THRUMIN1-YFP fusion protein fully complemented the low-light-induced chloroplast movements and partially complemented the high-light avoidance response in *thrumin1-1* mutants (Figure 1B).

THRUMIN1 belongs to a family of proteins found in plants and animals that contain conserved C-terminal glutaredoxin-like and putative zinc-binding cysteine-rich domains (Figure 1D and Figure S1C) [12]. The N-terminal half of THRUMIN1 is proline rich and predicted to be intrinsically disordered (Figure 1E). Members of this protein family include mammalian GRXCR1 [9]. Mouse and human *grxcr1* mutants are deaf [9, 10] and have shorter hair cell stereocilia [9, 13]. In transfected hair cells, GRXCR1 localized in the vicinity of actin in stereocilia similar to the actin-bundling protein, ESPIN [9]. The glutaredoxin domain of GRXCR1 was proposed to deglutathionylate actin [10], but a direct association between GRXCR1 and actin filaments has not been determined. The glutaredoxin-like domain of THRUMIN1 lacks the N-terminal catalytic cysteine conserved in dithiol and monothiol glutaredoxins (Figure S1D) [14]. Moreover, a fusion protein in which the only conserved cysteine was mutated to a serine in the glutaredoxin-like domain (THRUM1^{C231S}-YFP) complemented the chloroplast movement defect of *thrumin1* mutants as effectively as THRUM1-YFP (Figure 1B). These results indicate that THRUMIN1-mediated deglutathionylation is not necessary for chloroplast movement.

In *Arabidopsis*, THRUMIN1-YFP localized to filamentous structures (Figure 2; Movie S2; Movie S3). Treatment of leaf sections expressing THRUMIN1-YFP with 10 μM latrunculin B (LatB) disrupted THRUMIN1-YFP filament localization, resulting in a punctate localization pattern (Figure 2). These aggregates of THRUMIN1-YFP probably represent stable LatB-resistant actin filaments or multimeric THRUMIN1 (Figure 2). These results demonstrate that the filamentous localization pattern of THRUMIN1-YFP is actin dependent. As further evidence of an in vivo association between THRUMIN1 and actin, THRUMIN1-YFP localized in proximity to a CFP-mTalin actin reporter [15] in tobacco mesophyll cell transient expression assays (Figure S2A).

THRUMIN1 protein was previously identified in highly purified plasma membrane fractions isolated from *Arabidopsis* leaf tissue using mass spectrometry [16]. Furthermore, THRUMIN1 was predicted to be N-myristoylated and S-palmitoylated at glycine-2 and cysteine-3, respectively [17]. In the *thrumin1-1* mutants, glycine-2 is changed to a glutamic acid (Figure 1D), which is predicted to disrupt both N-myristoylation and S-palmitoylation and thereby preclude anchoring at the plasma membrane. To test these predictions, we expressed THRUMIN1^{G2A}-YFP and THRUMIN1^{C3F}-YFP fusion proteins in *thrumin1-1* mutants. Unlike THRUMIN1-YFP, both

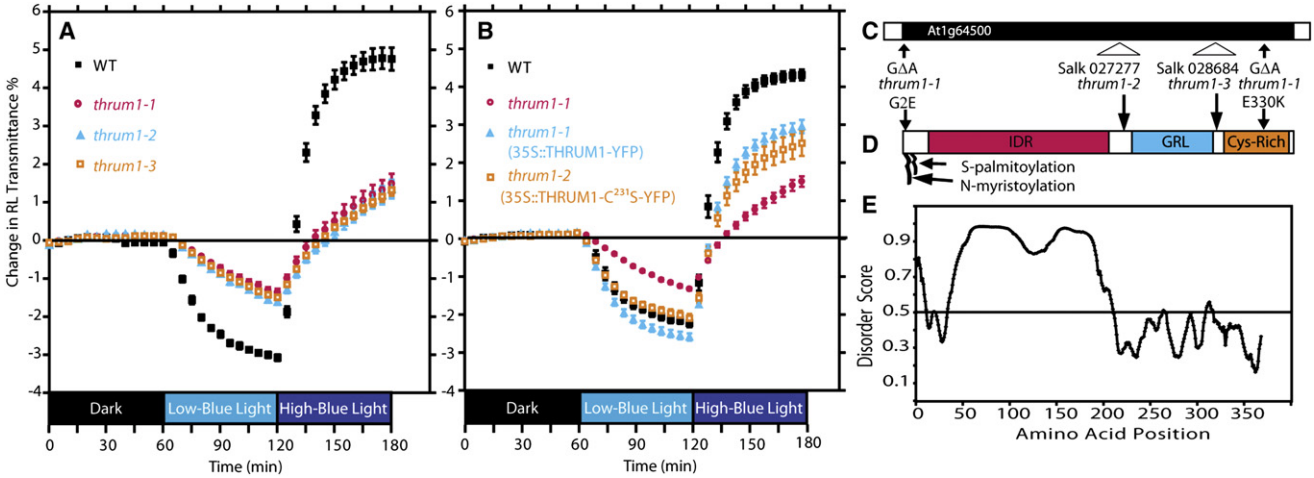

Figure 1. THRUMIN1 Is Required for Normal Chloroplast Movement

(A and B) Chloroplast movement-dependent changes in red-light transmittance through rosette leaves of wild-type (WT), *thrumin1* mutants, and transgenic lines in response to low (1.0 μmol m^{-2} s^{-1} turned on at 60 min) and high (40 μmol m^{-2} s^{-1} turned on at 120 min) blue light. Data represent the average ± standard error of the mean (SEM) (n = 6–7).

(C) Diagram of the At1g64500 locus, containing no introns, indicating the positions of the *thrumin1-1*^{G2E} and *thrumin1-1*^{E330K} mutations and the T-DNA insertion sites in *thrumin1-2* and *thrumin1-3* mutants.

(D) Protein schematic depicting the relative positions of the expected amino acid substitutions associated with the *thrumin1-1* mutant, the predicted N-myristoylation and S-palmitoylation sites, the intrinsically disordered region (IDR), the glutaredoxin like domain (GRX-like), and the cysteine-rich domain.

(E) VSL2 disorder prediction of THRUMIN1 indicating that the N-terminal half is disordered (score > 0.5). See also Figure S1 and Movie S1.

THRUMIN1^{G2A}-YFP and THRUMIN1^{C3F}-YFP failed to complement the chloroplast movement defect of *thrumin1-1* mutants (Figure S2B). Furthermore, THRUMIN1^{G2A}-YFP (Figure 2) and THRUMIN1^{C3F}-YFP (data not shown) localized diffusely in the cytoplasm surrounding the chloroplasts and other organelles. The diffuse distribution of THRUMIN1^{G2A}-YFP in the cytoplasm was not affected by LatB (Figure 2), which may signify that THRUMIN1's ability to interact with F-actin in *Arabidopsis* is dependent on anchoring to the plasma membrane. However, THRUMIN1^{G2A}-YFP colocalized with the CFP-mTalin actin reporter along thick actin cables when transiently expressed in tobacco (Figure S2A).

To test the significance of the intrinsically disordered region (IDR), the glutaredoxin-like (GRL) domain, and the Cys-rich domain, we expressed a series of N- and C-terminal truncations in planta. When stably transformed into *Arabidopsis*, the N-terminal IDR-GRL-YFP and IDR-YFP truncation fusion proteins localized to filaments (Figure 2) but failed to complement the chloroplast movement defect (Figure S2B). LatB treatment disrupted IDR-GRL-YFP and IDR-YFP filament localization, resulting in a diffuse plasma membrane localization pattern (Figure 2). The GRL-Cys-rich-YFP and Cys-rich-YFP fusion proteins displayed cytoplasmic localization when transiently expressed in tobacco mesophyll cells (Figure S2C). Together, these results demonstrate that the acylated N-terminal half, which contains the intrinsically disordered region of THRUMIN1, is sufficient for actin-dependent filament localization at the plasma membrane, whereas the Cys-rich domain is necessary for chloroplast movement.

We examined changes in the localization of THRUMIN1-YFP in response to blue light (Figures 3A and 3B; Movie S4). Treatment with a blue-light microstripe stimulated the chloroplast avoidance response (Figure 3A) and intensified THRUMIN1-YFP fluorescence along filaments inside the region irradiated with blue light (Figure 3B). THRUMIN1-YFP localization to filaments became less distinct, and the intensity of the YFP signal decreased, within 5 min after turning off the blue light (Figures 3A and 3B). The light-induced changes in THRUMIN1-YFP filament formation are reversible through subsequent light-dark treatments (Movie S4). THRUMIN1-YFP fluorescence intensity did not increase in response to a blue-light microstripe in *phot1phot2* double mutants (Figures 3A and 3C; Movie S4), demonstrating that the blue-light-mediated intensification of THRUMIN1-YFP fluorescence is dependent upon the phototropin blue-light photoreceptors. Similar to their redundant control over chloroplast movements, PHOT1 and PHOT2 function redundantly in this process because *phot1* (Figures 3A and 3D; Movie S4) and *phot2* (Figures 3A and 3D; Movie S4) single mutants displayed blue-light-mediated increases in THRUMIN1-YFP fluorescence. Recently, Kadota et al. [7] observed a meshwork of short actin filaments accumulating along the leading edges of chloroplasts in response to microbeam irradiation. Using plants expressing the LIFEACTIN-YFP actin reporter construct [18], we routinely observed an abundance of blue-light-independent thick cytoplasmic actin cables in mesophyll cells, but we have not observed a meshwork of actin around chloroplasts (Movie S5).

To test whether THRUMIN1 binds to actin filaments directly, we incubated purified recombinant protein with actin filaments and sedimented them at high speed (Figure 4A). In the absence of actin, THRUMIN1 did not sediment, whereas a substantial amount of THRUMIN1 pelleted in the presence of F-actin. A well-characterized actin-binding protein, *Arabidopsis* FIMBRIN1 [19], behaved in a similar manner, whereas GST failed to sediment in the presence of actin filaments (Figure 4A). These results indicate a direct interaction of THRUMIN1 with actin filaments. To test whether THRUMIN1 could bind more than one actin filament and/or generate higher-order structures, we performed low-speed filament sedimentation assays (Figure 4B). Under these conditions, actin filaments on their own do not sediment appreciably and remain in the supernatant. In the presence of THRUMIN1 or FIMBRIN1, a significant

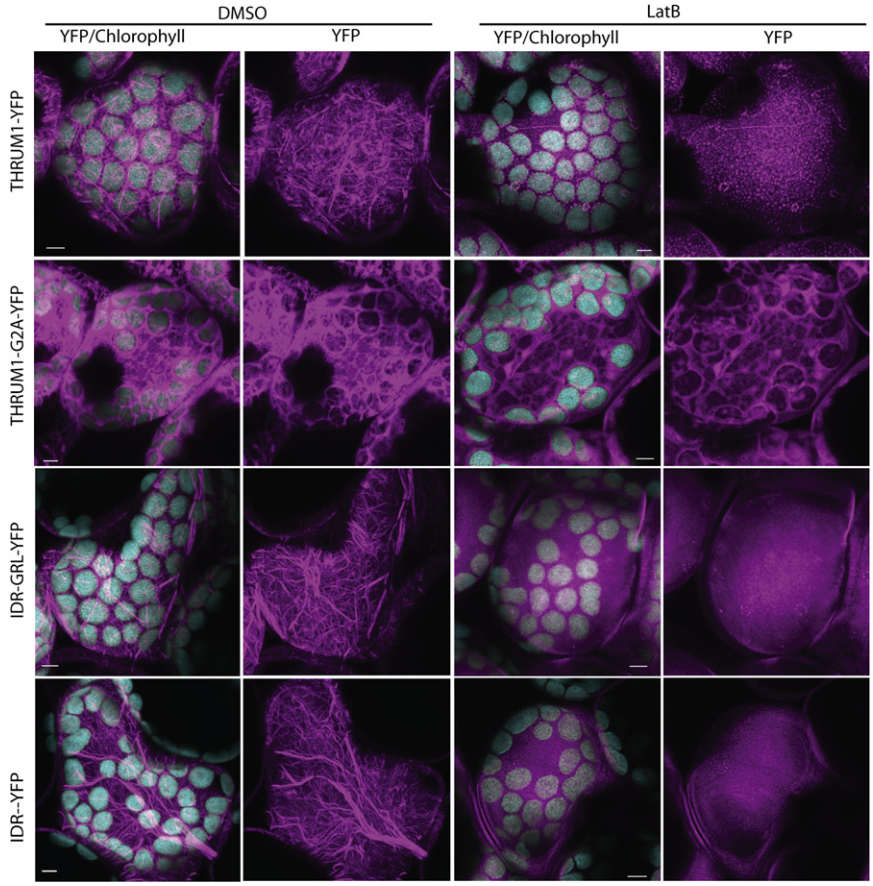

Figure 2. THRUMIN1 Localization to Filaments Is Actin Dependent, and Localization to the Plasma Membrane Is Dependent upon Residues Predicted to be Acylated

Localization of THRUM1-YFP, THRUM1^{G2A}-YFP, IDR-GRL-YRP, IDR-YFP (false-colored magenta), and chloroplasts (false-colored cyan) in *Arabidopsis* mesophyll cells infiltrated and mounted in 200 mM mannitol. To assay the effect of LatB on fusion protein localization, we incubated leaf sections in 0.5% dimethyl sulfoxide or 10 μM LatB for 1 hr prior to imaging. All scale bars represent 5 μm. See also Figure S2, Movie S2, and Movie S3.

amount of actin was found in the pellets, indicating the formation of higher-order structures that can be sedimented at low g forces. The amount of actin pelleted in the presence of THRUMIN1 was dose dependent and saturable (Figures 4C and 4D). Fluorescence light microscopy revealed that THRUMIN1 induced the formation of actin filament bundles (Figure 4G) similar to those produced by the well-characterized bundling protein, *Arabidopsis* VILLIN1 (Figure 4F). These results demonstrate unambiguously that THRUMIN1 is an F-actin binding and bundling protein in vitro.

THRUMIN1 belongs to a poorly understood family of proteins conserved in plants and animals [12]. Mutations in *Arabidopsis THRUMIN1* and mammalian homologs of *GRXCR1* [9, 10] result in impaired responses to environmental stimuli. Our finding that THRUMIN1 binds to and bundles actin in vitro establishes that members of this family of proteins can function as actin-accessory proteins.

Many actin-accessory proteins are involved in regulating actin dynamics at the plasma membrane in response to extracellular stimuli in animals. In plants, however, very little is known about the relationship between receptors at the plasma membrane, signaling cascades, and actin organization or dynamics. Our data suggest a model in which THRUMIN1 is involved in the bundling of actin filaments at the plasma membrane in response to blue light perceived by the phototropin photoreceptors. The light-dependent actin bundling mediated by THRUMIN1 may in turn dynamically remodel actin filament arrays nucleated by CHUP1 at the chloroplast outer envelope to drive chloroplast movements. Altogether, this study reveals that THRUMIN1 is a key link between light perception at the plasma membrane and the regulation of cortical actin dynamics.

Experimental Procedures

Detailed materials and methods are described in the Supplemental Experimental Procedures. Briefly, *Arabidopsis* plants were grown at ~23°C with cool-white florescent light (~100 μmol m^{-2} s^{-1}) and a 10 hr photoperiod. Chloroplast movements were monitored photometrically [20] or microscopically using blue-light microbeam irradiations. Live-cell imaging of palisade leaf cells was accomplished using a laser scanning inverted five-channel confocal microscope. THRUMIN1 actin binding and bundling were studied using low- and high-speed actin cosedimentation assays [19, 21]. The actin-bundling activity of THRUMIN1 was also observed by wide-field fluorescence microscopy using rhodamine-phalloidin-labeled actin [22, 23].

Supplemental Information

Supplemental Information includes two figures, Supplemental Experimental Procedures, and five movies and can be found with this article online at doi:10.1016/j.cub.2010.11.059.

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References

1. Kasahara, M., Kagawa, T., Oikawa, K., Suetsugu, N., Miyao, M., and Wada, M. (2002). Chloroplast avoidance movement reduces photodamage in plants. Nature *420*, 829–832.

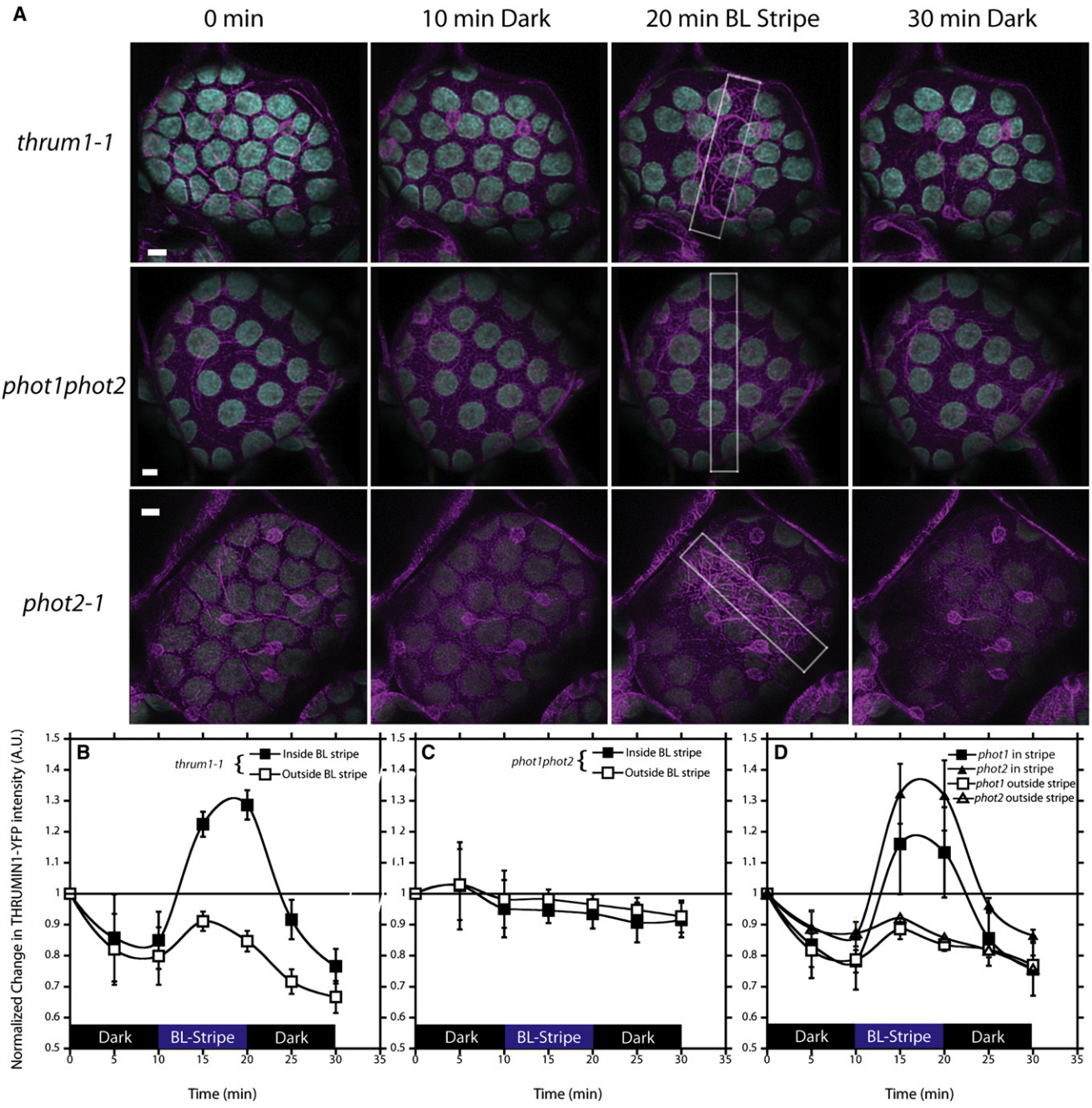

Figure 3. Light-Dependent THRUMIN1-YFP Localization to Filaments Is Modulated by the Phototropins

(A) THRUMIN1-YFP localization in low-light-acclimated *thrumin1-1*, *phot1-5*, *phot2-1*, and *phot1phot2* mutants in response to darkness and a blue-light microstripe. Areas within the white boxes were illuminated with a blue-light microstripe. Scale bars represent 5 μm.

(B–D) Quantification of THRUMIN1-YFP fluorescence over time within and outside of the region illuminated with the blue-light microstripe in *thrumin1-1* mutants (B), *phot1phot2* double mutants (C), and *phot1-5* and *phot2-1* single mutants (D). Data represent the average ± SEM (n = 3–5). See also Movie S4 and Movie S5.

2. Kagawa, T., Sakai, T., Suetsugu, N., Oikawa, K., Ishiguro, S., Kato, T., Tabata, S., Okada, K., and Wada, M. (2001). Arabidopsis NPL1: A phototropin homolog controlling the chloroplast high-light avoidance response. Science *291*, 2138–2141.

3. Sakai, T., Kagawa, T., Kasahara, M., Swartz, T.E., Christie, J.M., Briggs, W.R., Wada, M., and Okada, K. (2001). Arabidopsis nph1 and npl1: Blue light receptors that mediate both phototropism and chloroplast relocation. Proc. Natl. Acad. Sci. USA *98*, 6969–6974.

4. Jarillo, J.A., Gabrys, H., Capel, J., Alonso, J.M., Ecker, J.R., and Cashmore, A.R. (2001). Phototropin-related NPL1 controls chloroplast relocation induced by blue light. Nature *410*, 952–954.

5. Malec, P., Rinaldi, R.A., and Gabrys, H. (1996). Light-induced chloroplast movements in Lemna trisulca. Identification of the motile system. Plant Sci. *120*, 127–137.

6. Sun, S.X., Walcott, S., and Wolgemuth, C.W. (2010). Cytoskeletal cross-linking and bundling in motor-independent contraction. Curr. Biol. *20*, R649–R654.

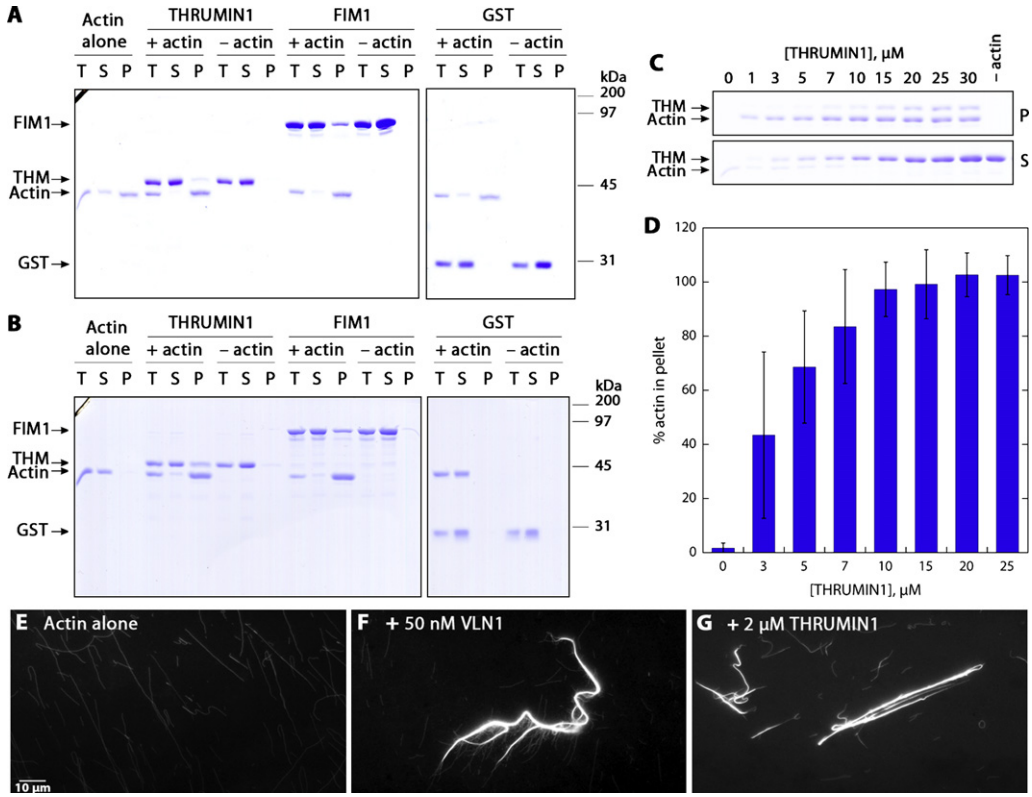

Figure 4. THRUMIN1 Binds to and Bundles F-Actin

(A) The ability of THRUMIN1 to bind to actin filaments was determined with high-speed cosedimentation assays. Actin (3 μM) was incubated with or without THRUMIN1 (THM), FIMBRIN1 (FIM1), or GST (10 μM) under polymerizing conditions and then sedimented at 186,000 × g. Samples of the total (T) reaction mix, supernatant (S), and pellet (P) from each condition were subjected to SDS-PAGE.

(B) The ability of THRUMIN1 to form higher-order structures with F-actin was determined with low-speed cosedimentation assays. Prepolymerized actin (3 μM) was incubated with or without THRUMIN1, FIM1, and GST (10 μM) under polymerizing conditions, followed by centrifugation at 13,500 × g, and T, S, and P samples were analyzed by SDS-PAGE.

(C and D) THRUMIN1 exhibits dose-dependent bundling of F-actin. The low-speed cosedimentation assay was performed with 3 μM F-actin and various amounts of THRUMIN1. Supernatant (bottom) and pellet (top) samples were subjected to SDS-PAGE (C). The percentage of actin in the pellet was determined by densitometry and plotted against the amount of THRUMIN1 (D). Data represent the average ± standard deviation.

(E–G) THRUMIN1 forms F-actin bundles. Reactions containing repolymerized actin (1 μM) alone (E), with 50 nM *Arabidopsis* VILLIN1 (VLN1), a known bundling protein (F), or with 2 μM THRUMIN1 (G) were decorated with rhodamine-phalloidin and imaged by fluorescence microscopy.

7. Kadota, A., Yamada, N., Suetsugu, N., Hirose, M., Saito, C., Shoda, K., Ichikawa, S., Kagawa, T., Nakano, A., and Wada, M. (2009). Short actin-based mechanism for light-directed chloroplast movement in Arabidopsis. Proc. Natl. Acad. Sci. USA *106*, 13106–13111.

8. Suetsugu, N., Yamada, N., Kagawa, T., Yonekura, H., Uyeda, T.Q., Kadota, A., and Wada, M. (2010). Two kinesin-like proteins mediate actin-based chloroplast movement in Arabidopsis thaliana. Proc. Natl. Acad. Sci. USA *107*, 8860–8865.

9. Odeh, H., Hunker, K.L., Belyantseva, I.A., Azaiez, H., Avenarius, M.R., Zheng, L.L., Peters, L.M., Gagnon, L.H., Hagiwara, N., Skynner, M.J., et al. (2010). Mutations in Grxcr1 are the basis for inner ear dysfunction in the pirouette mouse. Am. J. Hum. Genet. *86*, 148–160.

10. Schraders, M., Lee, K., Oostrik, J., Huygen, P.L.M., Ali, G., Hoefsloot, L.H., Veltman, J.A., Cremers, F.P.M., Basit, S., Ansar, M., et al. (2010). Homozygosity mapping reveals mutations of GRXCR1 as a cause of autosomal-recessive nonsyndromic hearing impairment. Am. J. Hum. Genet. *86*, 138–147.

11. DeBlasio, S.L., Luesse, D.L., and Hangarter, R.P. (2005). A plant-specific protein essential for blue-light-induced chloroplast movements. Plant Physiol. *139*, 101–114.

12. Navrot, N., Gelhaye, E., Jacquot, J.P., and Rouhier, N. (2006). Identification of a new family of plant proteins loosely related to glutaredoxins with four CxxC motives. Photosynth. Res. *89*, 71–79.

13. Beyer, L.A., Odeh, H., Probst, F.J., Lambert, E.H., Dolan, D.F., Camper, S.A., Kohrman, D.C., and Raphael, Y. (2000). Hair cells in the inner ear of the pirouette and shaker 2 mutant mice. J. Neurocytol. *29*, 227–240.

14. Meyer, Y., Buchanan, B.B., Vignols, F., and Reichheld, J.P. (2009). Thioredoxins and glutaredoxins: Unifying elements in redox biology. Annu. Rev. Genet. *43*, 335–367.

15. Saedler, R., Mathur, N., Srinivas, B.P., Kernebeck, B., Hülskamp, M., and Mathur, J. (2004). Actin control over microtubules suggested by DISTORTED2 encoding the Arabidopsis ARPC2 subunit homolog. Plant Cell Physiol. *45*, 813–822.

16. Alexandersson, E., Saalbach, G., Larsson, C., and Kjellbom, P. (2004). Arabidopsis plasma membrane proteomics identifies components of transport, signal transduction and membrane trafficking. Plant Cell Physiol. *45*, 1543–1556.

17. Boisson, B., Giglione, C., and Meinnel, T. (2003). Unexpected protein families including cell defense components feature in the N-myristoylome of a higher eukaryote. J. Biol. Chem. *278*, 43418–43429.

18. Riedl, J., Crevenna, A.H., Kessenbrock, K., Yu, J.H., Neukirchen, D., Bista, M., Bradke, F., Jenne, D., Holak, T.A., Werb, Z., et al. (2008). Lifeact: A versatile marker to visualize F-actin. Nat. Methods *5*, 605–607.

19. Kovar, D.R., Staiger, C.J., Weaver, E.A., and McCurdy, D.W. (2000). AtFim1 is an actin filament crosslinking protein from Arabidopsis thaliana. Plant J. *24*, 625–636.

20. Berg, R., Königer, M., Schjeide, B.M., Dikmak, G., Kohler, S., and Harris, G.C. (2006). A simple low-cost microcontroller-based photometric

instrument for monitoring chloroplast movement. Photosynth. Res. *87*, 303–311.

21. Huang, S., Robinson, R.C., Gao, L.Y., Matsumoto, T., Brunet, A., Blanchoin, L., and Staiger, C.J. (2005). Arabidopsis VILLIN1 generates actin filament cables that are resistant to depolymerization. Plant Cell *17*, 486–501.
22. Blanchoin, L., Amann, K.J., Higgs, H.N., Marchand, J.B., Kaiser, D.A., and Pollard, T.D. (2000). Direct observation of dendritic actin filament networks nucleated by Arp2/3 complex and WASP/Scar proteins. Nature *404*, 1007–1011.
23. Huang, S.J., Blanchoin, L., Kovar, D.R., and Staiger, C.J. (2003). Arabidopsis capping protein (AtCP) is a heterodimer that regulates assembly at the barbed ends of actin filaments. J. Biol. Chem. *278*, 44832–44842.