Figure S1. Uptake kinetics of plasma membrane–bound FM4-64 was observed by confocal microscopy in the For2A-GFP line (related to Fig. 1 C). (A) For2A-GFP line was pulse-labeled with 20 µM FM4-64 for 5 min. FM4-64 was gradually internalized. It took 30 min for FM4-64 to fully colocalize with For2A-GFP at the apex. At 3 h, most FM4-64 signal disappeared from the cell tip. (B) At 3 h, a significant amount of FM4-64 signal reached the vacuole. (C) At 6 h, the majority of FM4-64 signal existed solely on the vacuole. Bars, 5 µM.
Figure S2. Immunoblot using an anti-FLAG antibody of protein extracts from moss cells expressing the indicated constructs. (A) Related to Fig. 3. PTEND-FH1FH2-3XFLAG and HsPTEN-FH1FH2-3XFLAG do not complement the For2-RNAi phenotype, but are expressed. (B) Related to Fig. 5. 2XFYVE-FH1FH2-3XFLAG, TAPP1-FH1FH2-3XFLAG, and PH-FH1FH2-3XFLAG do not complement the For2-RNAi phenotype, but are expressed. Numbers are molecular weight standards in kD.
Figure S3. **Dose-dependent complementation of the formin RNAi phenotype using constructs that replace the For2A PTEN domain with PTEN homologues.**

Complementation using increasing amounts of the For2A (blue) expression plasmid, indicated below each bar in µg, shows a range of phenotypes. At low DNA concentrations, complementation is reduced both in area (top) and circularity (bottom). Between 5 and 15 µg of DNA optimum complementation is achieved. At higher concentrations, fewer plants are obtained from each transformation, indicative of toxicity possibly due to overexpression. Similar titrations with HsPTEN-FH1-FH2 (green) and PTEND-FH1-FH2 (yellow) show no response to increasing concentrations, indicating that these constructs are incapable of rescuing the phenotype. Conversely, PTENA-FH1-FH2 (purple) shows a similar trend as complementation with For2A. The images in Fig. 3 A are representative images of plants transformed with 5 µg of plasmid, which on average is the optimal concentration to achieve complementation. Error bars represent standard error.
Figure S4. Dose-dependent complementation of the formin RNAi phenotype using constructs that replace the For2A PTEN domain with unrelated PI(3,5)P₂-binding proteins. ATG18-FH1FH2 (light blue), MTM1-FH1FH2 (purple), and HsMTM1*-FH1FH2 (pink) all rescue the formin RNAi phenotype similarly to For2A (dark blue). Pictures used in Fig. 5 represent 5 µg of plasmid, which in all samples is the best optimum plasmid concentration. Plasmid concentrations in µg are indicated below each bar. Numbers above the bars indicate the number of plants measured for each condition. Error bars represent standard error.
Proteins that can functionally replace the For2A PTEN domain are not enriched at the cell apex. (A) GFP intensity in stable lines expressing GFP fusion constructs. Quantification of GFP levels in the medial section of cells expressing the GFP fusion constructs shown in Fig. 6. The levels of For2A PTEN-GFP (n = 5 cells), MTM1-GFP (n = 5 cells), and MTM1*GFP (n = 5 cells) are similar. PTENA-GFP (n = 4 cells) and PTEND-GFP (n = 4 cells) have higher levels of expression. Error bars represent standard deviation of the mean. (B) Quantification of chlorophyll autofluorescence shows that MTM1*FH1FH2-3XGFP complements For2 RNAi. Number of plants analyzed: 75, GUS-RNAi; 76, For2AB-5'UTR; 75, +For2A-3XFLAG; and 75, +MTM1*FH1FH2-3XGFP. Error bars are SEM and letters above the bars indicate statistical groups with α = 0.05 using an ANOVA analysis. (C) Representative images of plants expressing MTM1*FH1FH2-3XGFP or PTEN-3XFLAG-GFP. PTEN-3XFLAG-GFP is tip enriched, whereas MTM1*FH1FH2-3XGFP is cytosolic. Bar, 10 µm.
Video 1. For2A-GFP localizes to the cell tip and phragmoplast during cytokinesis (related to Fig. 1 A). Images are maximum intensity projections of a dividing cell expressing a functionally GFP-tagged copy of For2A. Images were acquired with a laser-scanning confocal microscope (model C1; Nikon). Frames were acquired every 90 s for 30 min. Bar, 5 µm. The video plays at 10 frames per second.

Video 2. Similar to For2A-GFP, For2A PTEN-GFP is recruited to the phragmoplast during cytokinesis (related to Fig. 1 A). Images are maximum intensity projections of a dividing cell stably expressing PTEN-GFP. Images were acquired with a laser-scanning confocal microscope (model C1; Nikon). Frames were acquired every 90 s for 42 min. The video plays at 10 frames per second. Bar, 5 µm.

Video 3. Colocalization of FM4-64–labeled membranes with For2A-GFP at the cell tip (related to Fig. 1 C). For2A-GFP line was pulse-labeled with 20 µM FM4-64 for 5 min and further incubated for 25 min at room temperature. The video was recorded after 30 min incubation. The dynamics of FM4-64–labeled membrane are similar to For2A-GFP at the cell tip. Images are from a single focal plane. Images were acquired with a laser-scanning confocal microscope (model C1; Nikon). Frames were acquired every 10 s for 12.6 min. The video plays at 20 frames per second. Bar, 5 µm.

Video 4. Imaging of For2A-GFP and actin in a growing protonemal cell (related to Fig. 2 A). Images are maximum intensity projections of a For2A-GFP cell stably expressing Lifeact-mCherry. Images were acquired with a spinning confocal microscope (model Ti-E, Nikon; equipped with a spinning disk head [model CSU-X1, Yokogawa Corporation of America]). Frames were acquired every 5 min for 45 min. The video plays at 5 frames per second. Bar, 5 µm.

Video 5. For2A-GFP generates actin filaments in vivo (related to Fig. 2 B). A burst of For2A-GFP signal, followed by a filamentous Lifeact-mCherry signal, was imaged in the For2A-GFP/LA-mCherry line. Images are from a single focal plane of a subapical region in a protonemal cell. Images were acquired with a spinning confocal microscope (model Ti-E, Nikon; equipped with a spinning disk head [model CSU-X1, Yokogawa Corporation of America]). Frames were acquired every 8 s for 56 s. The video plays at 5 frames per second. Bar, 5 µm.

Video 6. For2A-GFP localizes to dynamic cortical dots. The For2A PTEN domain mediates this localization. GFP fusions of PTENA, MTM1, and MTM1*, all proteins that when fused to the FH1-FH2 domains of For2 replace the function of the For2A PTEN domain, also form dynamic cortical dots. In contrast, GFP fusion of PTEND, which is unable to replace the function of the For2A PTEN domain, is diffuse at the cell cortex. These data are related to Fig. 6 C. Images were continuously acquired for at least 30 s with 50-msec exposure time (except for PTEND-GFP, which had 100-msec exposure time) for each frame using VAEM (model Ti-E, Nikon; equipped with TIRF). Video plays at 10 frames per second.

Video 7. Density of For2A-GFP cortical dots is reduced in silenced FAB1-RNAi plants. These data are related to Fig. 7 C. Images were continuously acquired for at least 30 s with 66.7-msec exposure time for each frame using VAEM (model Ti-E, Nikon; equipped with TIRF). Video plays at 10 frames per second.

Video 8. Linear For2A-GFP trajectories are actin dependent. (left) A population of cortical For2A-GFP dots moves along linear trajectories (related to top images in Fig. 8 A). (right) Latrunculin B treatment abolishes the linear population of cortical For2AGFP dots. Data are related to top images in Fig. 8 B. Images were continuously acquired for at least 30 s with 100-msec exposure time for each frame using VAEM (Nikon Ti-E body equipped with TIRF). Video plays at 10 frames per second.
Video 9. **For2A-GFP spot is found at the end of a growing actin filament (related to Fig. 9 A).** This longer time-lapse also shows that several formin spots move along preexisting filaments. The three panels are, from left to right, For2A-GFP, Lifeact-mCherry, and merge. In the merge, For2A-GFP and Lifeact-mCherry are false-colored magenta and cyan, respectively. Images were continuously acquired for at least 30 s with 80-msec exposure time for each frame using dual-view VAEM (Nikon Ti-E body equipped with TIRF). Video plays at 10 frames per second.

Video 10. **For2A-GFP dot glides along a preexisting actin filament (related to Fig. 9 B).** The three panels are, from left to right, For2A-GFP, Lifeact-mCherry, and merge. In the merge, For2A-GFP and Lifeact-mCherry are false-colored magenta and cyan, respectively. Images were continuously acquired for at least 30 s with 80-msec exposure time for each frame using dual-view VAEM (Nikon Ti-E body equipped with TIRF). Video plays at 10 frames per second.

### Table S1. Sequence comparison between formin PTEN domains and PTEN homologues

|            | For2A   | For2B   | PTENA   | PTENB   | PTENC   | PTEND   |
|------------|---------|---------|---------|---------|---------|---------|
| HsPTEN     | 24.5 / 15.4 | 26.1 / 16.0 | 27.0 / 18.9 | 26.3 / 17.6 | 20.0 / 13.4 | 16.0 / 10.8 |
| For2A      | 93.0 / 89.5 | 21.6 / 11.5 | 21.6 / 12.5 | 17.7 / 10.3 | 14.8 / 8.8  |
| For2B      | 20.4 / 11.4 | 20.7 / 12.1 | 16.2 / 9.3  | 14.9 / 9.4  |
| PTENA      | 86.0 / 81.4 | 41.8 / 31.8 | 39.7 / 29.4 |         |
| PTENB      | 43.3 / 32.5 | 41.0 / 30.4 |         |
| PTENC      | 51.5 / 43.6 |         |

Red = % similarity; green = % identity.
| Primer name | Primer sequence | Use |
|-------------|-----------------|-----|
| attB8 BamHI mEGFP | GGGGACAACCTTTGTATAACAAAGTGTGGGATCAGTGA-GCAAGGGCGAG | mCherry-L5L2 |
| attB2mEGFP BglII | GGGGACCACTTTGTACACGAGAATGCTGGGATGAGATGTTGTTTCCTTTTACTTCAGG | mCherry-L5L2 |
| GST-FOR: EcoRI | TTAAAGATCTGCTTGTTAAGCTGGTCTGTCGATTCTGACT | pET21-GST |
| GST-REV: EcoRI | TTAACTGCGGCACTGGCATCGACTTGGTTGCTGACT | pET21-GST |
| PTEN-REV: EcoRI | TTAACTGCGGCACTGGCATCGACTTGGTTGCTGACT | PTEN-GST |
| attB5BamHI mEGFP | GGAGTGCTTCATGACAAAATGCTGGGATGAGATGTTGTTTCCTTTTACTTCAGG | PTEN-GST |
| attB1 PTENA For | GGGGACAACCTTTGTACACGAGAATGCTGGGATGAGATGTTGTTTCCTTTTACTTCAGG | PTENa-pENT |
| attB1 PTEND For | GGGGACAACCTTTGTACACGAGAATGCTGGGATGAGATGTTGTTTCCTTTTACTTCAGG | PTENp-pENT |
| attB1 PTENA For | GGGGACAACCTTTGTACACGAGAATGCTGGGATGAGATGTTGTTTCCTTTTACTTCAGG | PTENa-pENT |
| attB1 PTEND For | GGGGACAACCTTTGTACACGAGAATGCTGGGATGAGATGTTGTTTCCTTTTACTTCAGG | PTENp-pENT |
| attB1 PTENA For | GGGGACAACCTTTGTACACGAGAATGCTGGGATGAGATGTTGTTTCCTTTTACTTCAGG | PTENa-pENT |
| attB1 PTEND For | GGGGACAACCTTTGTACACGAGAATGCTGGGATGAGATGTTGTTTCCTTTTACTTCAGG | PTENp-pENT |
| attB1 PTENA For | GGGGACAACCTTTGTACACGAGAATGCTGGGATGAGATGTTGTTTCCTTTTACTTCAGG | PTENa-pENT |
| attB1 PTEND For | GGGGACAACCTTTGTACACGAGAATGCTGGGATGAGATGTTGTTTCCTTTTACTTCAGG | PTENp-pENT |
| attB1 PTENA For | GGGGACAACCTTTGTACACGAGAATGCTGGGATGAGATGTTGTTTCCTTTTACTTCAGG | PTENa-pENT |
| attB1 PTEND For | GGGGACAACCTTTGTACACGAGAATGCTGGGATGAGATGTTGTTTCCTTTTACTTCAGG | PTENp-pENT |
| attB1 PTENA For | GGGGACAACCTTTGTACACGAGAATGCTGGGATGAGATGTTGTTTCCTTTTACTTCAGG | PTENa-pENT |
| attB1 PTEND For | GGGGACAACCTTTGTACACGAGAATGCTGGGATGAGATGTTGTTTCCTTTTACTTCAGG | PTENp-pENT |
| attB1 PTENA For | GGGGACAACCTTTGTACACGAGAATGCTGGGATGAGATGTTGTTTCCTTTTACTTCAGG | PTENa-pENT |
| attB1 PTEND For | GGGGACAACCTTTGTACACGAGAATGCTGGGATGAGATGTTGTTTCCTTTTACTTCAGG | PTENp-pENT |
| attB1 PTENA For | GGGGACAACCTTTGTACACGAGAATGCTGGGATGAGATGTTGTTTCCTTTTACTTCAGG | PTENa-pENT |
| attB1 PTEND For | GGGGACAACCTTTGTACACGAGAATGCTGGGATGAGATGTTGTTTCCTTTTACTTCAGG | PTENp-pENT |
| attB1 PTENA For | GGGGACAACCTTTGTACACGAGAATGCTGGGATGAGATGTTGTTTCCTTTTACTTCAGG | PTENa-pENT |
| attB1 PTEND For | GGGGACAACCTTTGTACACGAGAATGCTGGGATGAGATGTTGTTTCCTTTTACTTCAGG | PTENp-pENT |
| attB1 PTENA For | GGGGACAACCTTTGTACACGAGAATGCTGGGATGAGATGTTGTTTCCTTTTACTTCAGG | PTENa-pENT |
| attB1 PTEND For | GGGGACAACCTTTGTACACGAGAATGCTGGGATGAGATGTTGTTTCCTTTTACTTCAGG | PTENp-pENT |
| attB1 PTENA For | GGGGACAACCTTTGTACACGAGAATGCTGGGATGAGATGTTGTTTCCTTTTACTTCAGG | PTENa-pENT |
| attB1 PTEND For | GGGGACAACCTTTGTACACGAGAATGCTGGGATGAGATGTTGTTTCCTTTTACTTCAGG | PTENp-pENT |

**Table S2. Primers used in this study**