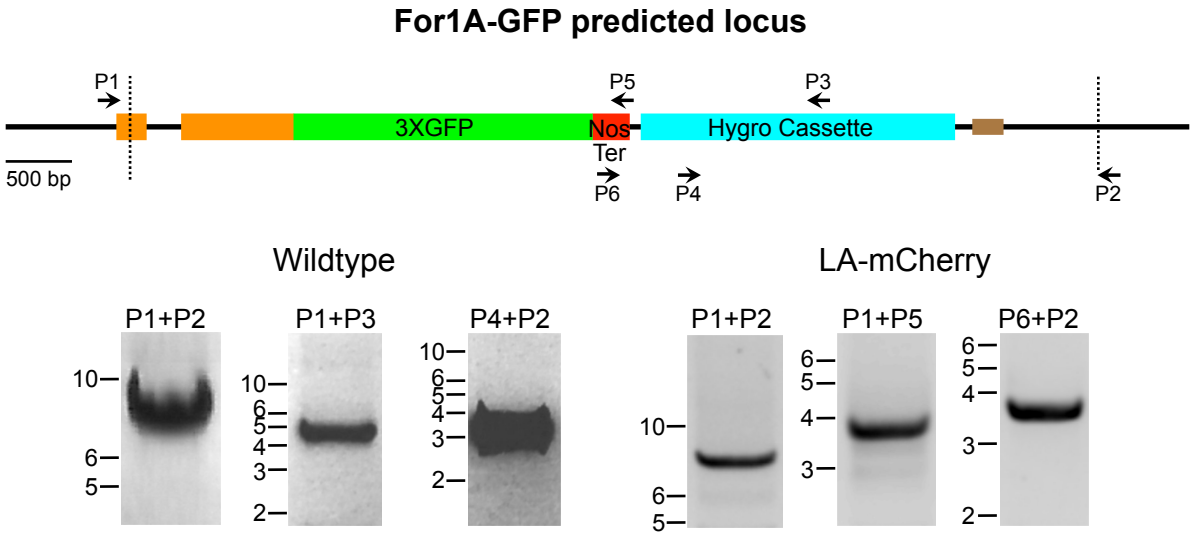


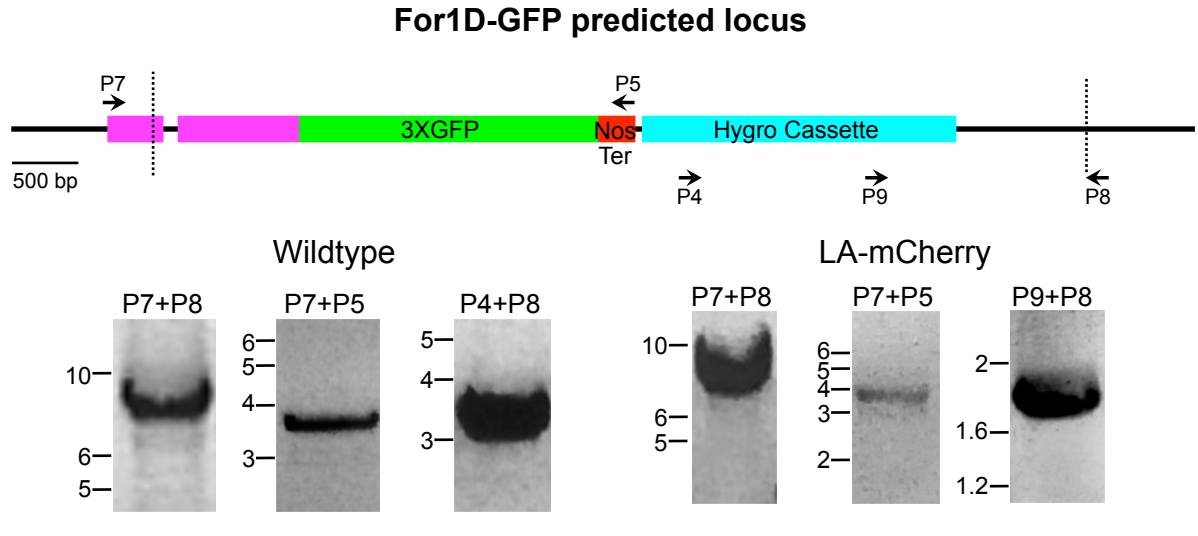


Figure S1. Molecular characterization of endogenous tagging of For1A (top) and For1D (bottom). Diagrams illustrate the result of homologous recombination mediated insertion of 3XGFP sequences in the genomic locus of each of the formins. Coding exons are indicated by thick orange (For1A) or magenta (For1D) boxes and For1A 3' untranslated exon is indicated by a thin brown box. The 3' untranslated sequences for For1D were replaced by the inserted sequences. Inserted sequences (3XGFP, NOS terminator, and hygromycin resistance cassette) are indicated by thick colored boxes. The dashed lines indicate the junction between the knock-in construct and upstream and downstream genomic sequences. Small arrows above and below the diagrams represent primers used for genotyping. Scale bar is 500 bp.'PCR products obtained with the indicated primer pairs using the template DNA isolated from the indicated moss line were separated on an agarose gel and stained with ethidium bromide. Molecular weight is indicated in kb. Predicted sizes are as follows: For1A (P1+P2), 7274 bp represents a single insertion at the locus; For1A (P1+P3) in wild type, 5040 bp represents proper targeting at the 5' insertion site; For1A (P1+P5) in lifeact-mCherry, 3593 bp represents proper targeting at the 5' insertion site; For1A (P4+P2) in wild type, 3197 bp represents proper targeting at the 3' insertion site; For1A (P6+P2) in lifeact-mCherry, 3775 bp represents proper targeting at the 3' insertion site; For1D (P1+P2), 7218 bp represents a single insertion at the locus; For1D (P1+P3), 3600 bp represents proper targeting at the 5' insertion site; For1D (P4 +P2) in wild type, 3114 bp represents proper targeting at the 3' insertion site; For1D (P5+P2) in lifeact-mCherry, 1771 bp represents proper targeting at the 3' insertion site. All primers are listed in Table S1.

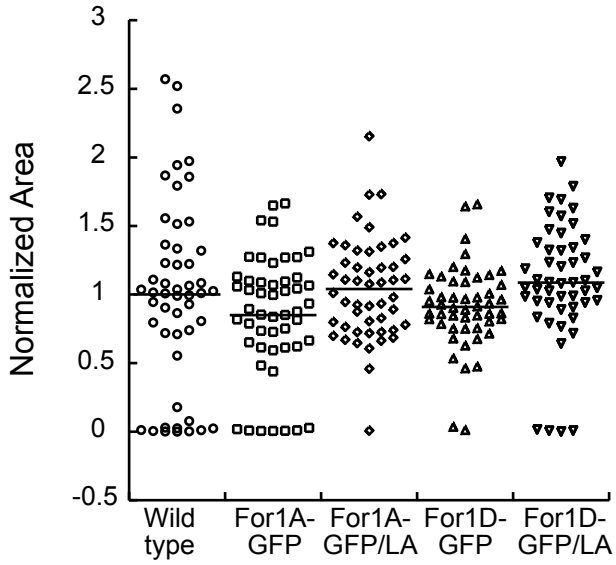


Figure S2. Tagging the For1A or For1D locus does not significantly alter plant growth. Plants of the indicated genotype were regenerated from protoplasts. After seven days, plants were imaged with a stereo fluorescent microscope to capture the chlorophyll autofluorescence, which is a proxy for plant area. The area of the chlorophyll autofluorescence, normalized to wild type plants for each experiment, is shown for each indicated genotype. ANOVA analysis with a Tukey HSD post hoc test did not find significant differences between any of the indicated genotypes.

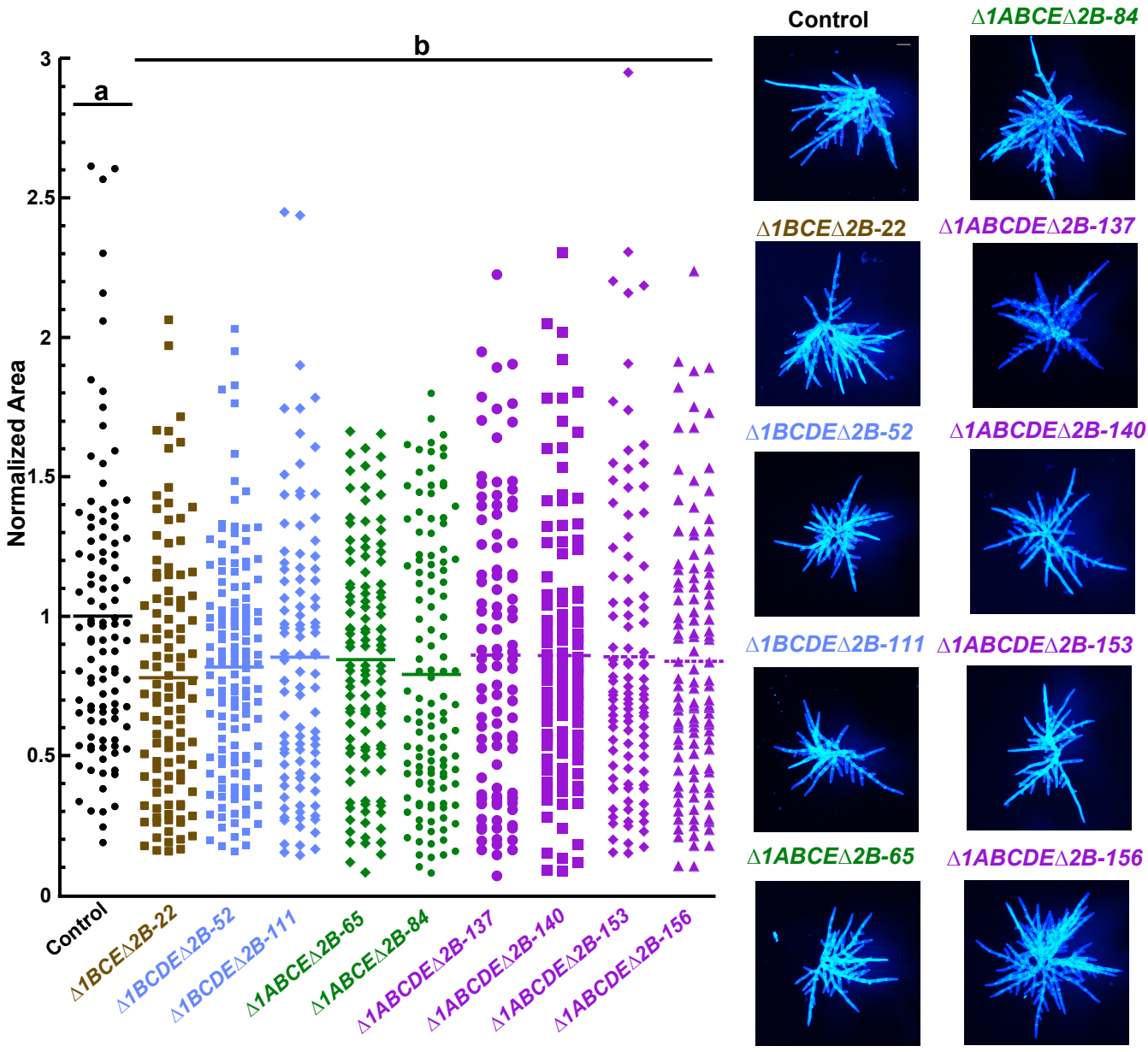
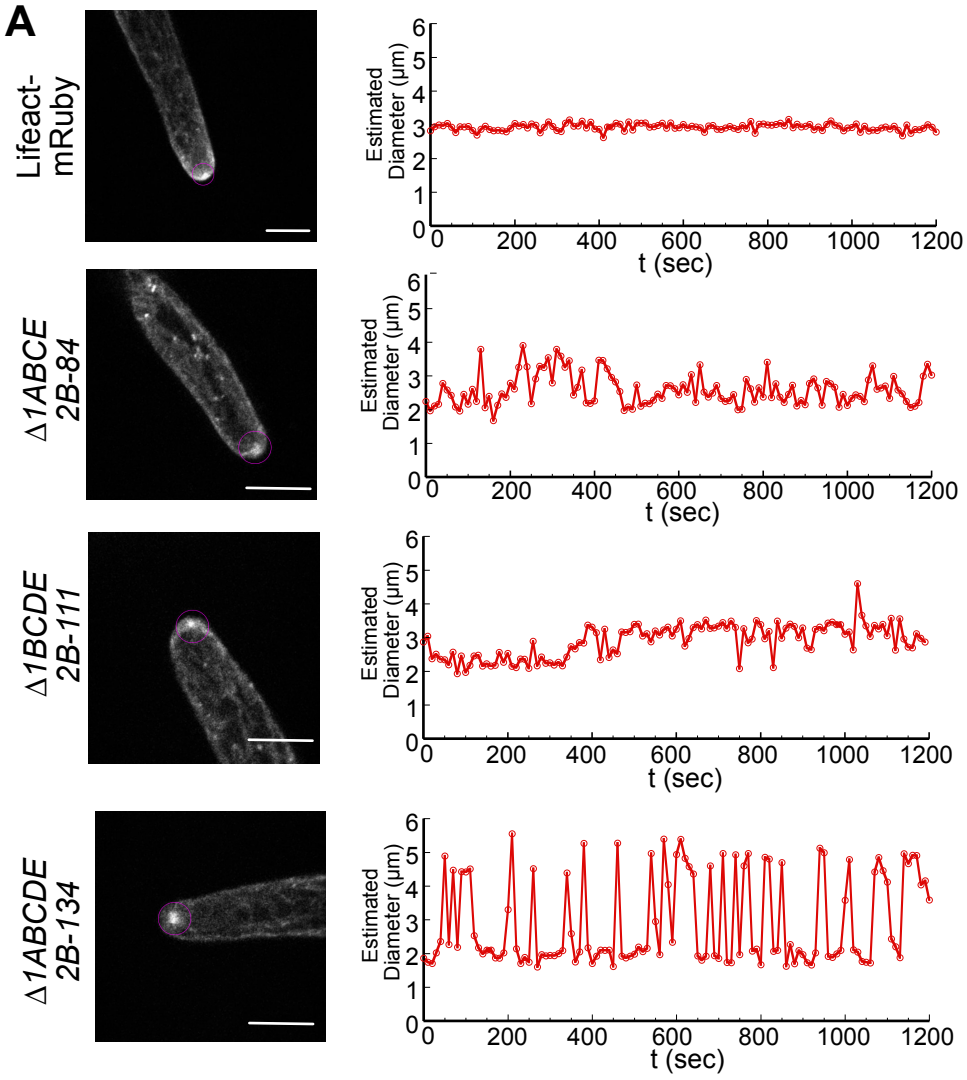
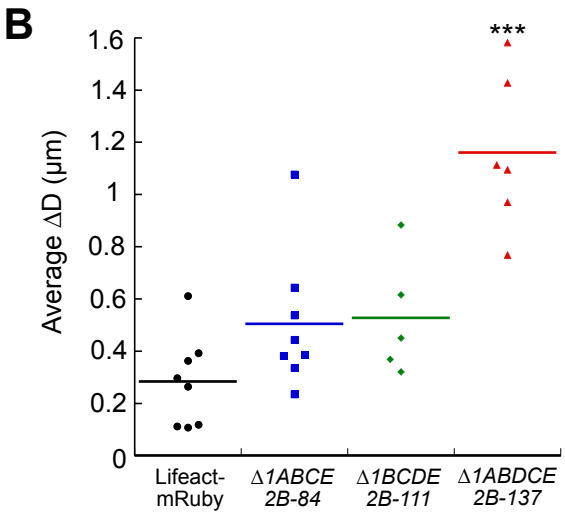


Figure S3. Characterization of formin null lines. Plants of the indicated genotypes (disruptions in each locus described in Table S2) were regenerated from protoplasts. After six-seven days, the plants were stained with Calcofluor White and images were taken to measure plant area, normalized to the average of the lifeact-mRuby control. ANOVA analysis with Fisher's LSD post hoc test found that all mutants are slightly smaller than the control, but they do not differ significantly from each other.





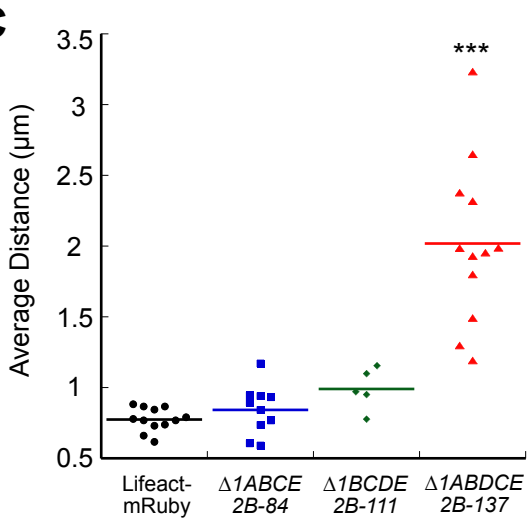
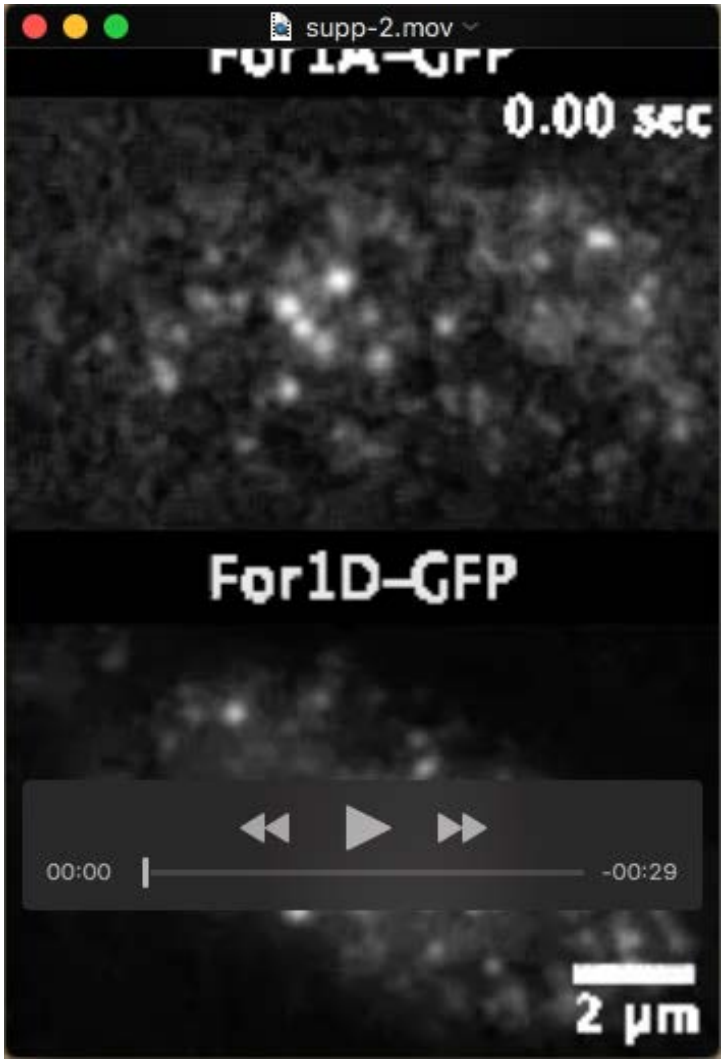
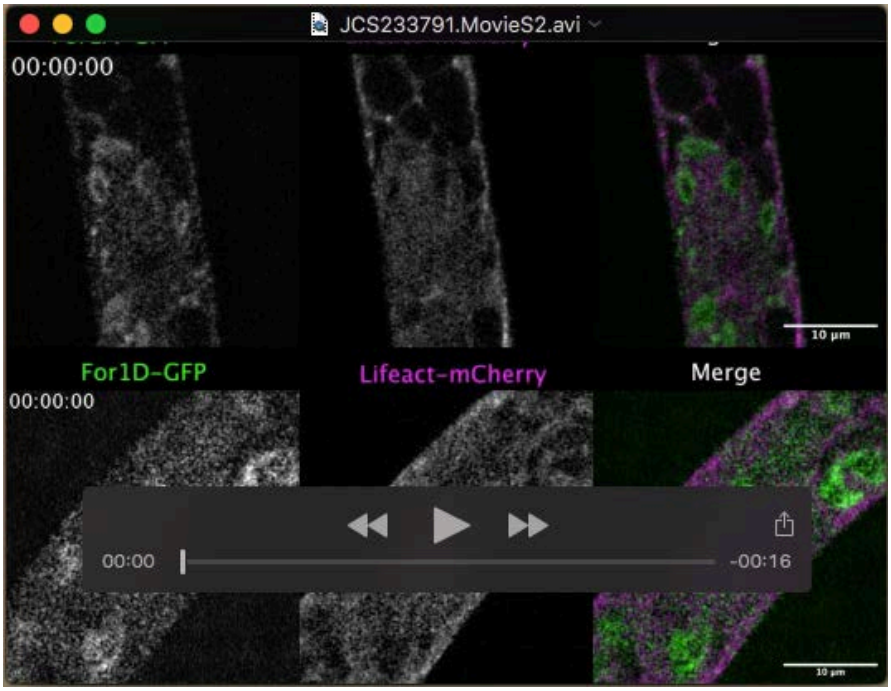


Figure S4. Quantification of cytoplasmic apical actin in formin null lines. (A) Selected images of lifeact-mRuby in control and formin null cell as described in Fig 4. Apical actin foci were tracked over time with Fiji plugin TrackMate within a 5µm- diameter circle (magenta circles on the images). The estimated diameter obtained from the tracking results were plotted over time. Scale bar, 10 µm. (B) The absolute difference of the estimated diameter between two neighboring time points (ΔD) over 20 minutes are averaged for each cell. (C) The average distance of the apical actin foci to the cell apex was measured using Fiji in each cell from 10-13 frames of the time-lapse acquisition spread out through the 20 minute acquisition time. An ANOVA with a Tukey HSD post hoc test was performed. *** indicates significance with $p<0.001$.

Supplemental Movies

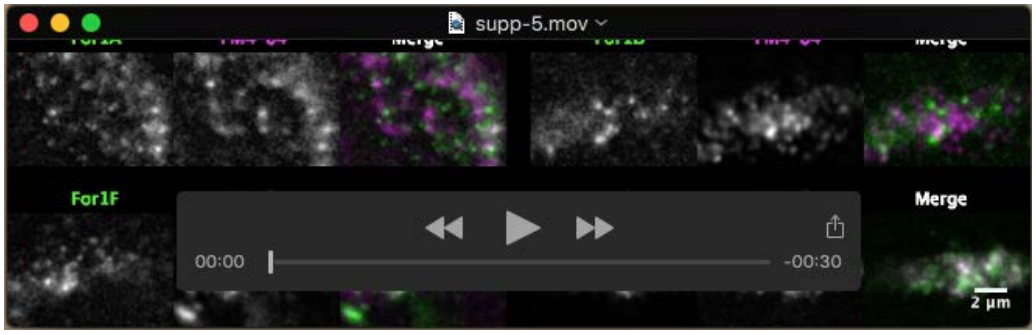


Movie 1. Cortical For1A-GFP and For1D-GFP imaged with VAEM. Time interval, 0.05 seconds. Scale bar, 2 µm. Video is playing at 20 fps. See also Fig1D.

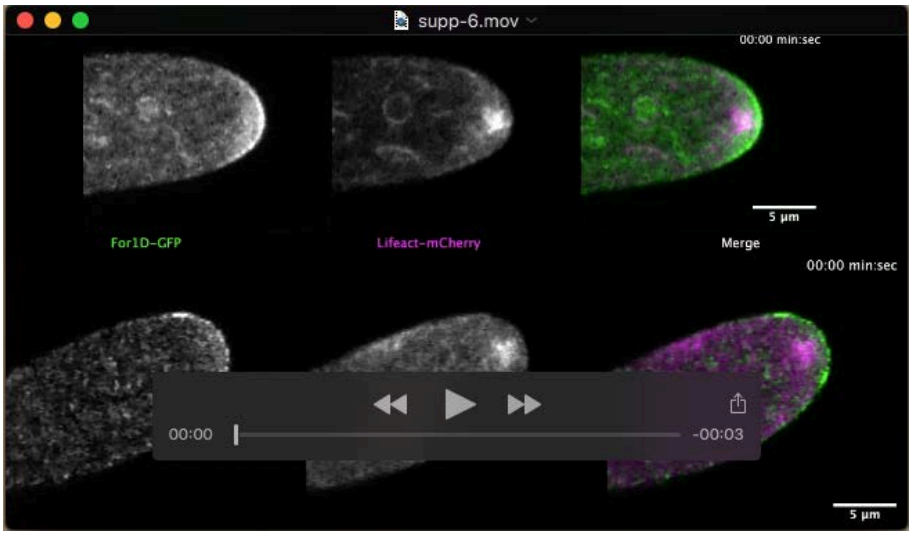


Movie 2. For1A-GFP or For1D-GFP (green) together with actin labeled with lifeact-mCherry (magenta) in wild-type cells. Images are from single focal planes acquired on a laser scanning confocal microscope. Time interval, 20 seconds. Video is playing at 10 fps. Time stamps represent hr:min:sec. Scale bars, 10 µm. See also Fig 2.

Movie 3. Plasma membrane and internal membrane structures labeled with FM4-64 (magenta) in wild-type cells expressing For1A-GFP or For1D-GFP (green). Images are from single focal planes acquired on a laser scanning confocal microscope. Time interval, 5 seconds. Video is playing at 20 fps. See also Fig 3A.

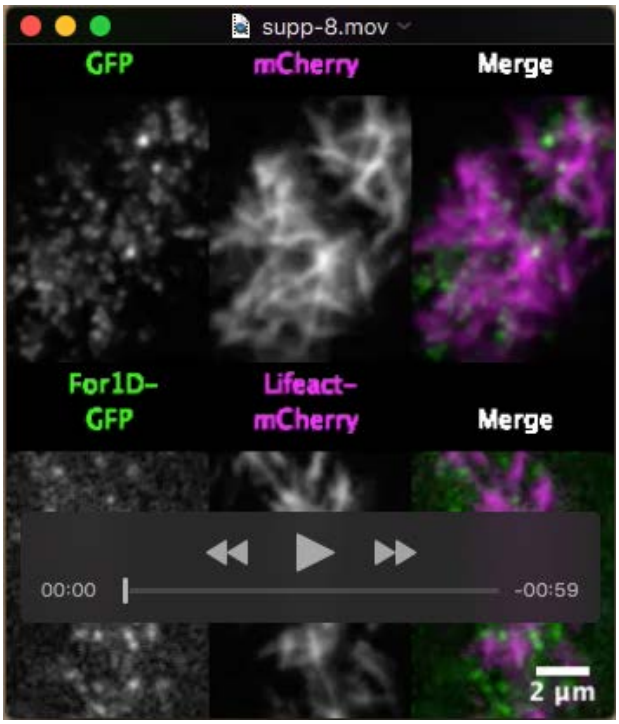


Movie 4. Cortical For1A-GFP or For1D-GFP (green) together with vesicles labeled with FM4-64 (magenta) imaged with VAEM. Time interval, 0.1 seconds. Scale bar, 2 µm. Video is playing at 10 fps. See also Fig 3B.



Movie 5. For1A-GFP or For1D-GFP (green) together with actin labeled with lifeact-mCherry (magenta) in wild-type cells. Images are from single focal planes acquired on a laser scanning confocal microscope. Time interval, 20 seconds. Video is playing at 10 fps. See also Fig 4A.

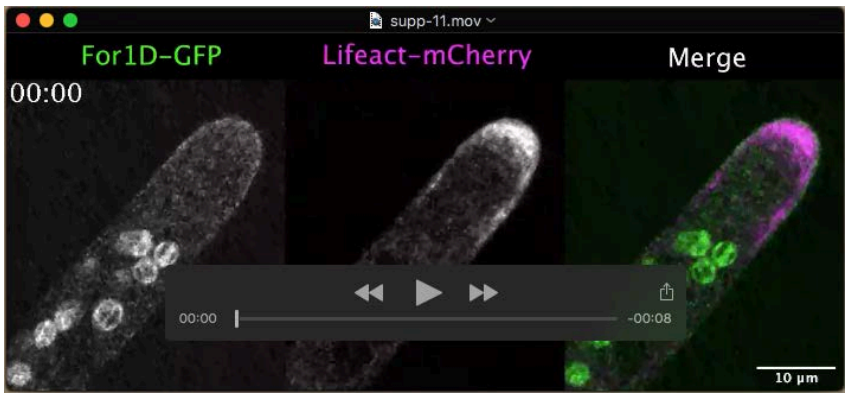
Movie 6. Apical actin structure in control and formin null lines labeled with lifeact-mRuby. Images are from maximum projections of three z-slices in the medial section of the cell acquired on a laser scanning confocal. Timer interval, 10 seconds. Video is playing at 10 fps. See also Fig 4B.



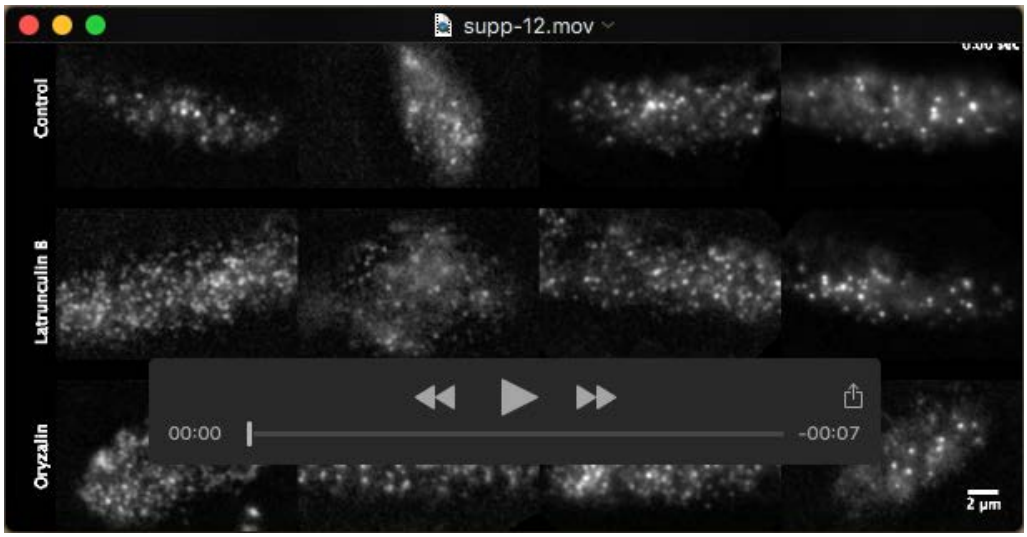
Movie 7. Cortical For1A-GFP and For1D-GFP (green) together with actin labeled with lifeact-mCherry (magenta) imaged with VAEM. Time interval, 0.1 seconds. Scale bar, 2 µm. Video is playing at 10 fps. See also Fig 5A.

Movie 8. Cortical actin labeled with lifeact-mRuby in control and formin null lines was imaged with VAEM. Time interval, 0.12 seconds. Scale bar, 2 µm. Video is playing at 20 fps. See also Fig 5B.

Movie 9. For1A-GFP (green) and actin labeled with Lifeact-mCherry (magenta) in a wild-type cell treated with 12.5 µm oryzalin. Images are from single focal planes acquired on a laser scanning confocal microscope. Time interval, 5 seconds. Video is playing at 8 fps. Time stamps represent min:sec. Scale bars, 10 µm. See also Fig 6.



Movie 10. For1D-GFP (green) and actin labeled with Lifeact-mCherry (magenta) in a wild-type cell treated with 12.5 µm oryzalin. Images are from single focal planes acquired on a laser scanning confocal microscope. Time interval, 5 seconds. Video is playing at 8 fps. Time stamps represent min:sec. Scale bars, 10 µm. See also Fig 6.



Movie 11. Cortical For1A-GFP, For1D-GFP, For1F-GFP and For2A-GFP under latrunculin B or oryzalin treatment imaged with VAEM. Time interval, 0.05 seconds. Scale bar, 2 µm. Video is playing at 20 fps. See also Fig 7.

Supplemental Tables

Table S1 Primers used in this study

Primer Name	Primer Sequence	Use
For1A-5-Tarm-F	GGGGACAAGTTTGTACAAAAAAGCAGGCTATTTAAATTCAATGGGAAACTTTCAGATCTCGG	Tag 5' end of For1A locus
For1A-5-Tarm-R	GGGGACAACTTTTGTATACAAAGTTGACGTTGTGGTGGTTGTCCTC	Tag 5' end of For1A locus
For1A3-Tarm-F	GGGGACAACTTTGTATAATAAAGTTGCTGGGGCGATGTTTGAAAATTA	Tag 3' end of For1A locus
For1A-3-Tarm-R	GGGGACCACTTTGTACAAGAAAGCTGGGTATTTAAATGAAAGCGAAGAGCAAGTGGT	Tag 3' end of For1A locus
For1A-3xGFP-gt-f (P1)	GAAGTCTTGGAGACGCTGGTCA	Genotyping For1A GFP knock-in
For1A-3xGFP-GT-R (P2)	TGCTCTCAGTCATTTCCCTTGC	Genotyping For1A GFP knock-in
For1D-5-Tarm-R	GGGGACAACTTTTGTATACAAAGTTGTATTCGTTTTATTCGGCAGGGAG	Tag 5' end of For1D locus
For1D-3-Tarm-F	GGGGACAACTTTGTATAATAAAGTTGCTTGCAGCATGATTTTGAAAGG	Tag 3' end of For1D locus
For1D-3-Tarm-R	GGGGACCACTTTGTACAAGAAAGCTGGGTATTTAAATAGAGCTCAAGGTTGCCAAAA	Tag 3' end of For1D locus
For1D-5Tarm-F-new	GGGGACAAGTTTGTACAAAAAAGCAGGCTATTTAAATGCGTTTGAGCGTCTTCAAGC	Tag 5' end of For1D locus
For1D-3xGFP-GT-F (P7)	CTCACAACATTGCCATCCAGCT	Genotyping For1D GFP knock-in
For1D-3xGFP-GT-R (P8)	ATTCGGGGACGCAATCGAGATT	Genotyping For1D GFP knock-in
Hyg-F (P3)	CTGTCGAGAAGTTTCTGATCG-	Genotyping 5' insertion site
Hyg-R (P4)	TCGGTTTCCACTATCGGC	Genotyping 3' insertion site
NOSter-jct-Rev (P5)	ATGCTTAACGTAATTCAACAG	Genotyping 5' insertion site
NOSter-F (P6)	CGTTCAAACATTTGGCAATAAAGTTTC	Genotyping 3' insertion site
35S-int-Rev-Seq (P9)	ACAGATAGCTGGGCAATGGA	Genotyping 3' insertion site
For1A-Cas9UP-attB1-F	GGGGACAAGTTTGTACAAAAAAGCAGGCTTACGGAATCTCCATGTGACCTTC	Clone 5' homology are of for1A for generating stop-cassette insertion

For1A-Cas9UP-attB4-R	GGGGACAACTTTGTATAGAAAAGTTGGGTGGGGCAAGGATAGGCTCAAC	Clone 5' homology are of for1A for generating stop-cassette insertion
For1A-Cas9DOWN-attB3-F	GGGGACAACTTTGTATAATAAAGTTGTAATGCGGTGGCTGGCGG	Clone 3' homology are of for1A for generating stop-cassette insertion
For1A-Cas9DOWN-attB2-R	GGGGACCACTTTGTACAAGAAAGCTGGGTTCCACATCCTGGCGAGGAGTG	Clone 3' homology are of for1A for generating stop-cassette insertion
For1D-cas9UP-attB1-F	GGGGACAAGTTTGTACAAAAAAGCAGGCTTAGACAGTGCTGGTGTAATTCG	Clone 5' homology are of for1D for generating stop-cassette insertion
For1D-Cas9UP-attB4-R	GGGGACAACTTTGTATAGAAAAGTTGGGTGGCATAAACCTCCACCGTC	Clone 5' homology are of for1D for generating stop-cassette insertion
For1D-Cas9DOWN-attB3-F	GGGGACAACTTTGTATAATAAAGTTGTACATGGGTTGGCATAGTAGG	Clone 3' homology are of for1D for generating stop-cassette insertion
For1D-Cas9DOWN-attB2-R	GGGGACCACTTTGTACAAGAAAGCTGGGTTTAGGGTTGTGCAAAGCTG	Clone 3' homology are of for1D for generating stop-cassette insertion
For1A-CRISPR-GT-Big-F	TCATCCAACTTGTCCACTTC	Genotype for1A mutant plants, amplify from genomic DNA outside of homology arm
For1A-CRISPR-GT-Big-R	CACTCTCTGGGATATTCTGAG	Genotype for1A mutant plants, amplify from genomic DNA outside of homology arm
For1D-CRISPR-GT-Big-F	AATCAACCACGGAGATCG	Genotype for1D mutant plants, amplify from genomic DNA outside of homology arm
For1D-CRISPR-GT-Big-R	CCTTCAATGTCTTCTCCCTG	Genotype for1D mutant plants, amplify from genomic DNA outside of homology arm
For1A-CRISPR-GT-F	TCCATCCGTCCTCAGAAG	Genotype for1A mutant plants
For1A-CRISPR-GT-R	AACGACTATCAAATGGTGCC	Genotype for1A mutant plants
For1D-CRISPR-GT-F	AGCTAAAGCTGTTGGACG	Genotype for1D mutant plants
For1D-CRISPR-GT-R	AGCCTTCTTGTACGCATTC	Genotype for1D mutant plants
For1A-CRISPR-F2	ccatGCCCATACTGTGTTTAGCAT	Protospacer for making for1A knockout

For1A-CRISPR-R2	aaacATGCTAAACACAGTATGGGC	Protospacer for making for1A knockout
For1A-CRISPR-F	ccatCTATCTTCGAACGCATGCGG	Protospacer for making for1A knockout
For1A-CRISPR-R	aaacCCGCATGCGTTCGAAGATAG	Protospacer for making for1A knockout
For1D-CRISPR-F	ccatTTATGCCGACGGTCCAGTGA	Protospacer for making for1D knockout
For1D-CRISPR-R	aaacTCACTGGACCGTCGGCATAA	Protospacer for making for1D knockout
For1D-CRISPR-F2	ccatGCAATCGGTATCTCATGGGT	Protospacer for making for1D knockout
For1D-CRISPR-R2	aaacACCCATGAGATACCGATTGC	Protospacer for making for1D knockout

Table S2 Summary of the Formin null mutants

Name	Background	Genomic mutation	Final protein
Lifeact-mRuby2	WT	NA	NA
Δ*for1BCE2B-22*	Lifeact-mRuby2	(Mallett et al., 2019)	(Mallett et al., 2019)
Δ*for1BCDE2B-52*	Δ*for1BCE2B-22*	For1D, 4bp deletion and 2 point mutations in exon2	58aa, 54aa identical to WT
Δ*for1BCDE2B-111*	Δ*for1BCE2B-22*	For1D, stop cassette insertion	18aa, 10aa identical to WT
Δ*for1ABCE2B-65*	Δ*for1BCE2B-22*	For1A, 14bp deletion in exon1	25aa, 21aa identical to WT
Δ*for1ABCE2B-84*	Δ*for1BCE2B-22*	For1A, stop cassette insertion	28aa, 20aa identical to WT
Δ*for1ABCDE2B-137*	Δ*for1BCDE2B-111*	For1A, stop cassette insertion	28aa, 20aa identical to WT
Δ*for1ABCDE2B-140*	Δ*for1BCDE2B-111*	For1A, stop cassette insertion	28aa, 20aa identical to WT
Δ*for1ABCDE2B-153*	Δ*for1ABCE2B-84*	For1D, stop cassette insertion	18aa, 10aa identical to WT
Δ*for1ABCDE2B-156*	Δ*for1ABCE2B-84*	For1D, stop cassette insertion	18aa, 10aa identical to WT