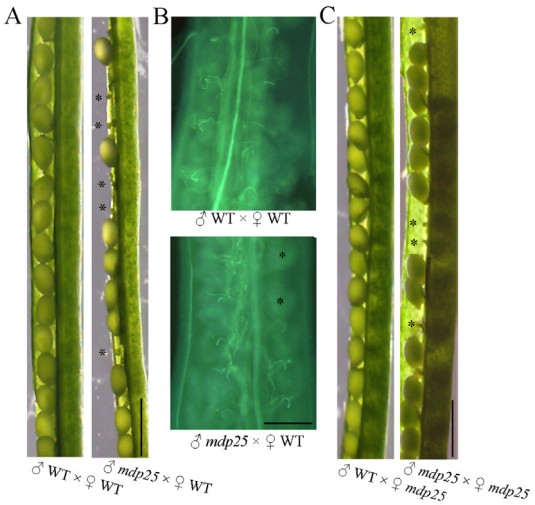
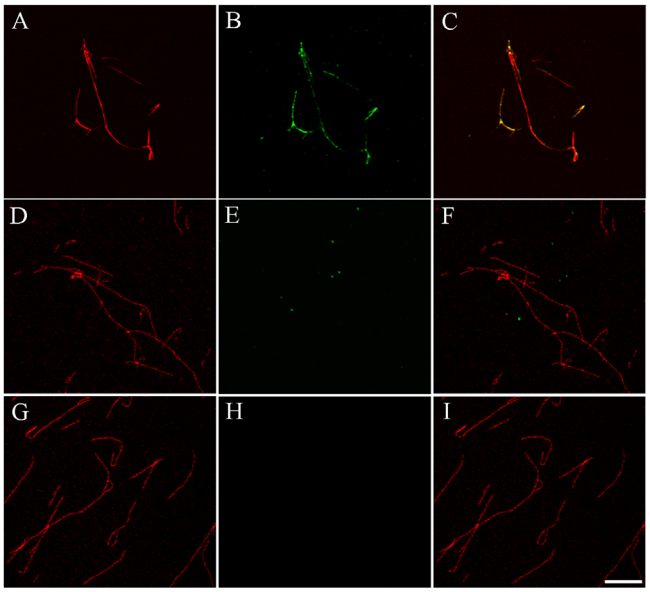


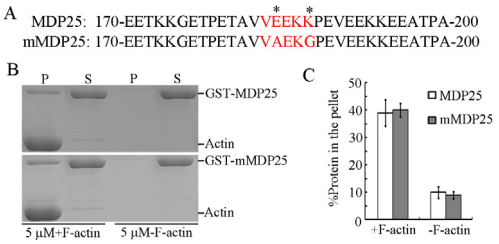
Supplemental Figure 1. The *MDP25* Loss-of-Function Mutant Affects Fertilization of Embryos and Pollen Tube Growth.

(A) A restricted amount of pollen grains from wild-type Col-0 and *mdp25-1* were used to pollinate wild-type Col-0 stigmata. Fertilized (green and mature) and aborted unfertilized (thin and barren; asterisks) ovules in dissected siliques. Bar = 1 mm. **(B)** The *mdp25-1* pollen tubes missed some micropyles (see asterisks). Bar = 125 μm. **(C)** Restricted amount of pollen grains from wild-type Col-0 and *mdp25-1* were used to pollinate *mdp25-1* stigmata. Fertilized (green and mature) and aborted unfertilized (thin and barren; asterisks) ovules in dissected siliques. Bar = 1 mm.



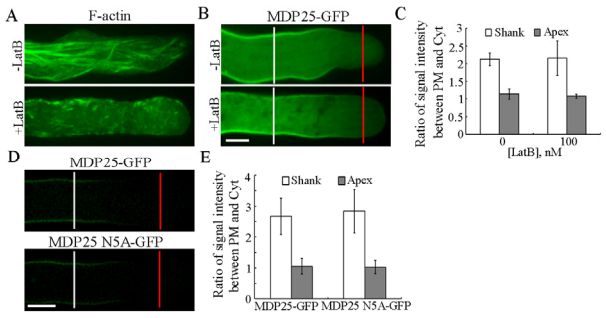
Supplemental Figure 2. MDP25 Colocalizes with F-actin in Vitro.

In vitro immunofluorescence microscopy showed that GST-MDP25 colocalizes with F-actin. Red fluorescence signals indicate Alexa 488–phalloidin-labeled F-actin whilst green signals indicate GST-MDP25 probed with anti-GST and TRITC-conjugated secondary antibodies. **(A)** actin filaments, **(B)** MDP25, and **(C)** merged image. **(D)** actin filaments, **(E)** denatured MDP25, and **(F)** merged image. **(G)** actin filaments, **(H)** secondary antibody alone, and **(I)** merged image. Bar in **(I)** = 10 μm for **(A)** to **(I)**.



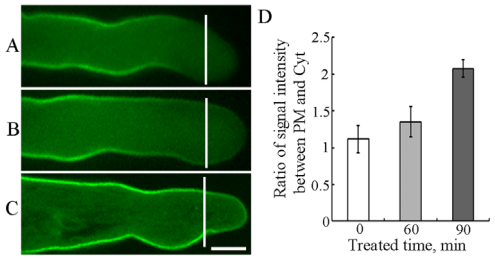
Supplemental Figure 3. Mutation of the Calcium-binding Site of MDP25 Does not Affect F-actin Binding.

(A) The residues of MDP25 and mutated MDP25 (mMDP25). Red font represents the one calcium-binding motif. Asterisks indicate mutated sites in the amino acid sequence of MDP25. **(B)** Co-sedimentation assay of mMDP25 binding to F-actin. Compared with wild-type MDP25, mutated MDP25 of calcium-binding sites does not affect MDP25 actin filament binding activity. Supernatant (S), Pellet (P). **(C)** Statistical analysis of data in **(B)**. The amount of protein in the pellets was estimated by gel density scanning, and is expressed as a percentage of total protein. Error bars represent mean ± SD (n = 3).



Supplemental Figure 4. Plasma Membrane Localization of MDP25 Is Independent of the Presence of Actin Filaments.

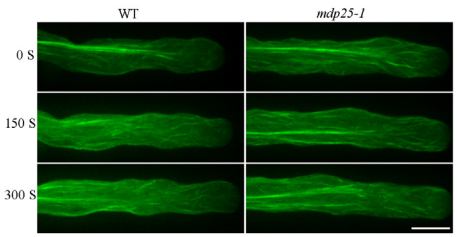
(A) Actin filaments were primarily disrupted in pollen tubes of the lifeact-mEGFP transgenic plant following treatment with 100 nM LatB for 5 min. **(B)** Mid-plane section of a MDP25-GFP pollen tube treated with 100 nM LatB for 5 min. MDP25-GFP was localized to the shank plasma membrane, but not to the subapical region, the flank, or the apex. White and red lines indicate the position where arbitrary units were measured across the pollen tube for the shank and apex, respectively. Scale bar = 5 μm. **(C)** Ratio of fluorescence intensities between the plasma membrane with cytosol across the pollen tube shanks (white line) and the pollen tube subapical region (red line) in **(B)**. More than 14 growing pollen tubes for each line were quantified. Error bars represent mean ± SD. **(D)** Mid-plane section of a MDP25 N5A-GFP pollen tube from P_{MDP25}: MDP25 N5A-GFP and a MDP25-GFP pollen tube from P_{MDP25}: MDP25-GFP transgenic plants in which MDP25 N5A-GFP and MDP25-GFP was primarily localized to the shank plasma membrane, but not to the subapical region, the flank, or the apex, respectively. Scale bar = 5 μm. **(E)** Ratio of fluorescence intensities between the plasma membrane with cytosol across the pollen tube shanks (white line) or across the pollen tube subapical region (red line) in **(D)**. More than eight growing pollen tubes for each line were quantified. Error bars represent mean ± SD.



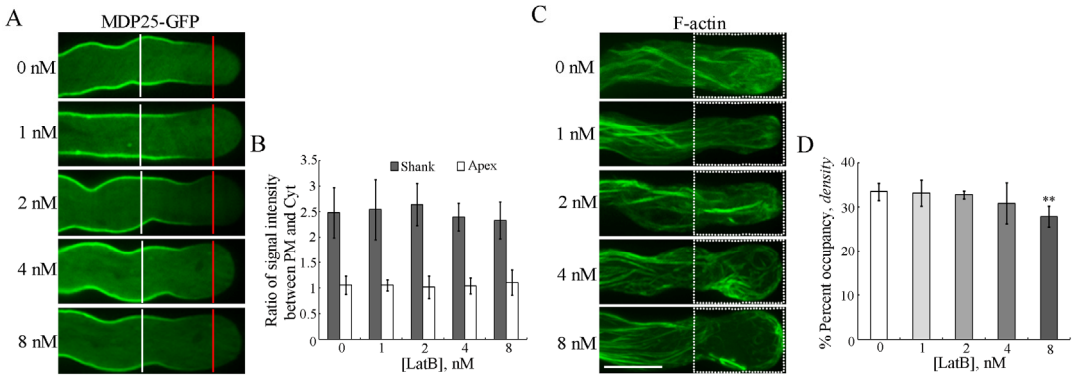
Supplemental Figure 5. Subcellular Localization of MDP25-GFP Is Affected When Tubes were Treated with Ca^{2+} Chelator.

Mid-plane section of a MDP25-GFP pollen tube treated with 1 mM BAPTA-AM for 0 min **(A)**, 60 min **(B)**, and 90 min **(C)** in which MDP25-GFP is localized to the plasma membrane of the subapical region, the flank, and the apex. The white lines indicate the position where arbitrary units were measured across the pollen tube tip. Bar = 5 μm.

(D) The ratio of fluorescence intensities between plasma membrane with cytosol across the pollen tube subapical region (white line) in **(A)-(C)**. More than 12 growing pollen tubes of each treatment were quantified. Error bars represent mean ± SD.



Supplemental Figure 6. Organization and Density of Actin Filaments Are Altered in the Subapical Region of Pollen Tubes of the *mdp25-1* Mutant. Time-lapse fluorescent images taken from Supplemental Movies 5 and 6 online, showing the organization and density of actin filaments were altered in the apical region, whilst there was no obvious difference in the shank region of the *mdp25-1* and wild-type pollen tubes. Bar = 10 μm



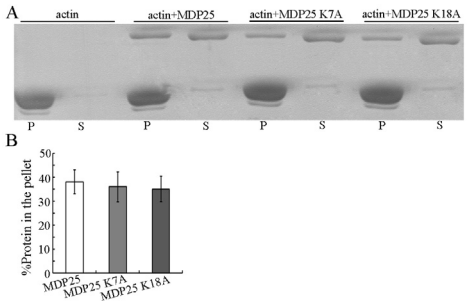
Supplemental Figure 7. Subcellular Localization of MDP25-GFP in Pollen Tubes Is not Altered by LatB Treatment.

(A) Mid-plane section of a MDP25-GFP pollen tube treated with various concentrations of LatB. MDP25-GFP is primarily localized to the shank plasma membrane, but not at the subapical region, the flank, or the apex. White and red lines indicate the position where arbitrary units were measured across the pollen tube, respectively.

(B) Fluorescence intensity ratios between plasma membrane with cytosol across the pollen tube shanks (white line) or across the pollen tube subapical region (red line) in a mid-plane section of a MDP25-GFP pollen tube treated with various concentrations of the LatB. More than 15 growing pollen tubes for each line were quantified. Error bars represent mean ± SD.

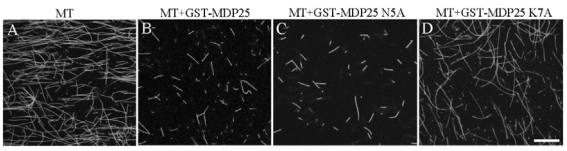
(C) The pollen tubes from *lifeact-mEGFP* transgenic plants were treated with various concentrations of LatB. The areas outlined with the white dots indicate the position where arbitrary units were measured across the pollen tube. Bar = 10 μm.

(D) Density (occupancy) analysis shown in (C) reveals that the actin filament density was decreased in the subapical region when pollen tubes were treated with 8 nM LatB for 30 min. More than 15 growing pollen tubes for each line were quantified. Error bars represent mean ± SD. (Student's *t*-test, **P < 0.01).



Supplemental Figure 8. Mutation of Two Sites of MDP25 Does not Affect It Targeting to Actin Filaments.

(A) A high-speed co-sedimentation assay was used to determine F-actin binding of MDP25 K7A and MDP25 K18A. An SDS-PAGE assay showed that, similar to wild-type MDP25, MDP25 K7A and MDP25 K18A mostly appeared in the supernatant (S) in the absence of F-actin, yet co-sedimented with F-actin in the pellets (P), respectively. **(B)** Statistical analysis for **(A)**. The amount of MDP25, MDP25 K7A, and MDP25 K18A in the pellets was estimated by gel density scanning, and is expressed as a percentage of total MDP25, MDP25 K7A, and MDP25 K18A, respectively. Error bars represent mean ± SD (n = 3).



Supplemental Figure 9. MDP25 N5A Destabilizes Microtubules in Vitro. Microtubules were polymerized in a 20 μM rhodamine-labeled tubulin solution **(A)** in the absence of GST-MDP25, **(B)** in the presence of 8 μM GST-MDP25, **(C)** in the presence of 8 μM GST-MDP25 N5A, and **(D)** in the presence of 8 μM GST-MDP25 K7A. Scale bar = 20 μm.