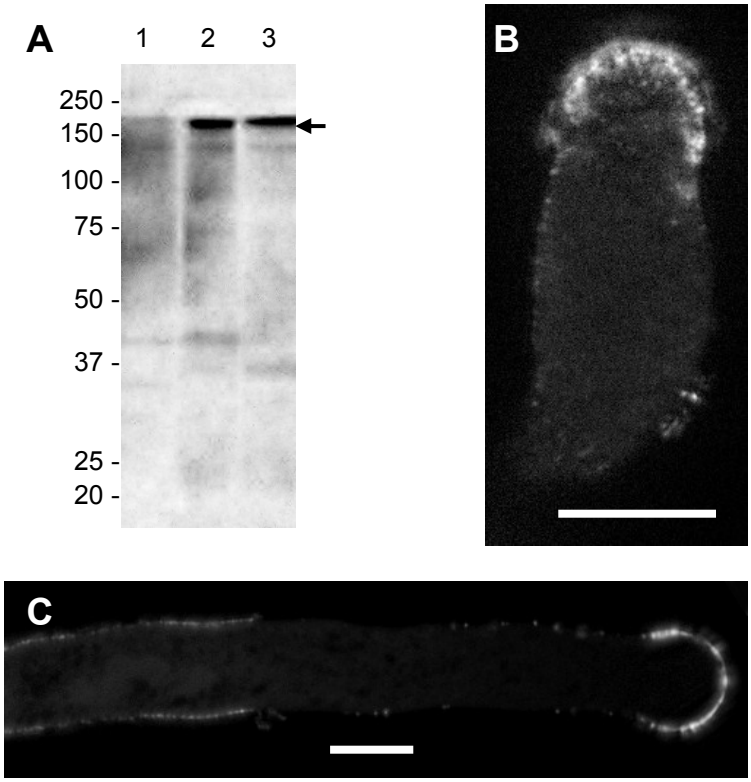


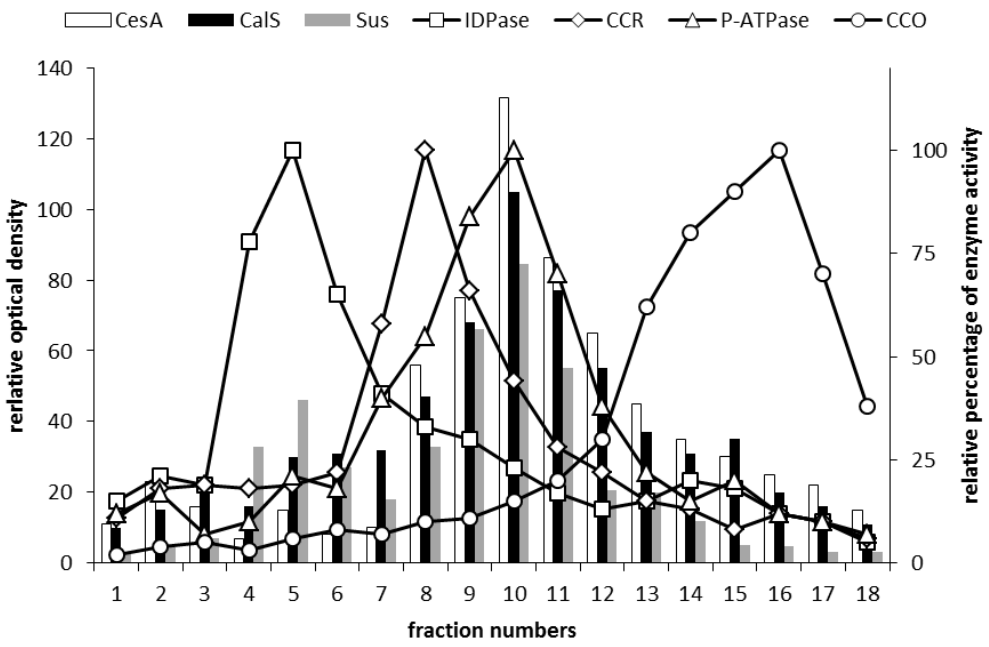
Supplemental figure 1

Characterization of the anti-CalS DDA antibody.

(A) Immunoblot assay of DDA antibody on cytoplasmic proteins (lane 1), membrane proteins (lane 2) from tobacco pollen tubes and on extracts from Arabidopsis flowers (lane 3). About 30 µg of proteins were loaded in each lane. The antibody cross-reacted with a polypeptide of 220-225 kD in the tobacco membrane fraction and in the Arabidopsis fraction (arrow). The blot is overexposed to detect additional cross-reacting bands. Molecular mass standards (expressed as kD) are on the left.

(B) Immunolocalization of CalS with the DDA antibody in short pollen tubes; labeling was mainly found in the apical domain.

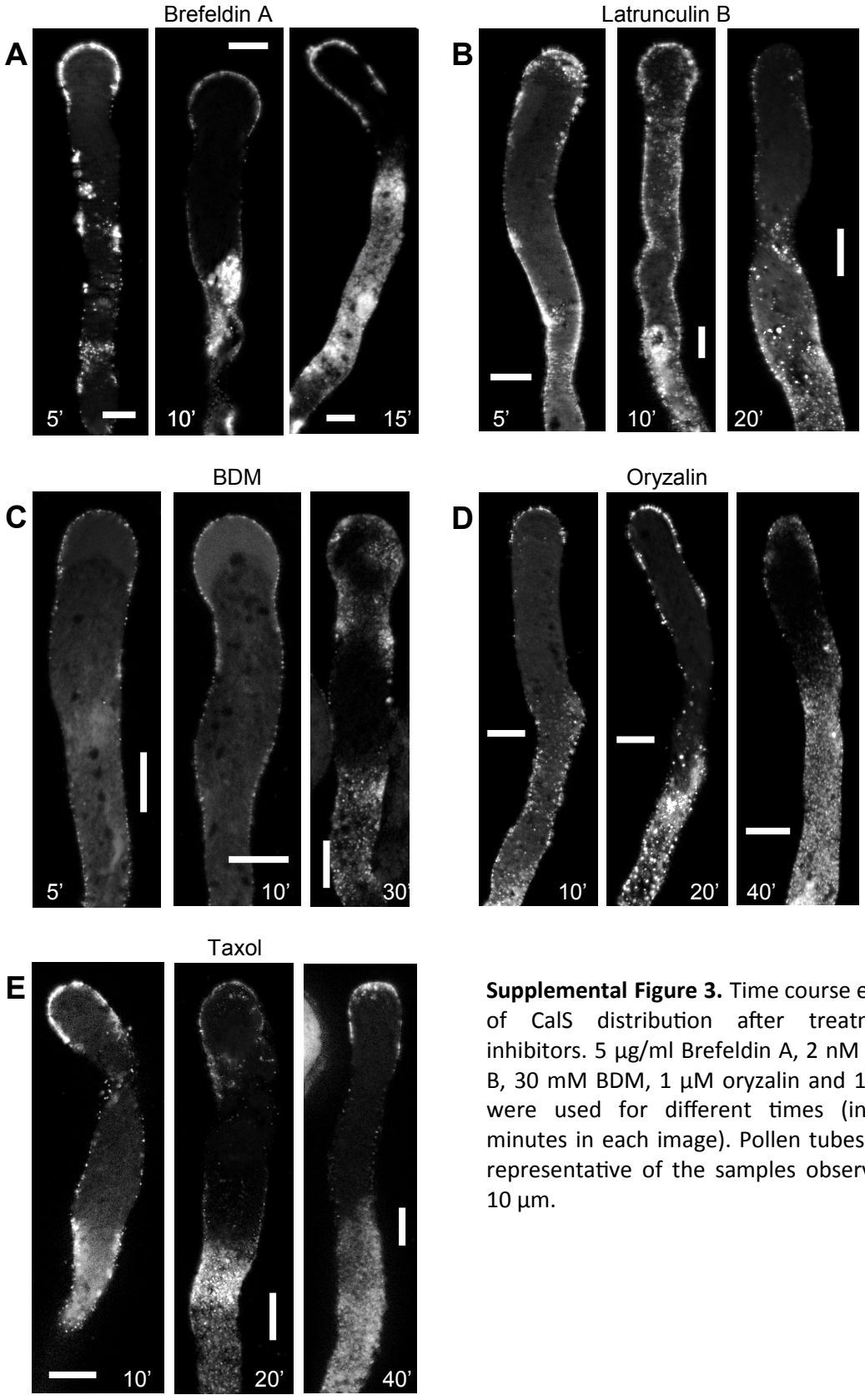
(C) Distribution of CalS as shown by DDA antibody in longer pollen tubes. Staining was detected in the apical region and in the distal segment of pollen tubes. Bars = 10 µm.



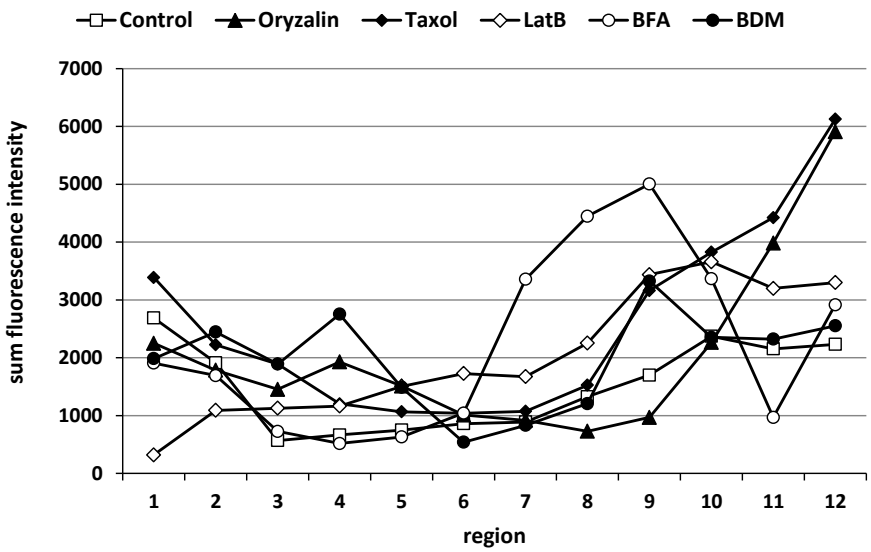
Supplemental figure 2

Relative quantitation profile of CesA, CalS, and Sus from three independent immunoblots performed on fractions of the sucrose density gradient centrifugation (vertical axis is on the left). Values were calculated using the Quantity One software; background was calculated separately for each blot. Relative quantity of enzymes is expressed as integrated density.

Relative activity of of enzyme markers specific for different cellular compartments is also reported for each fraction: IDPase for Golgi membranes, CCR for endoplasmic reticulum, P-ATPase for plasma membrane and CCO for mitochondria. Values are indicated as percentage of maximum enzyme activity on the right vertical axis.



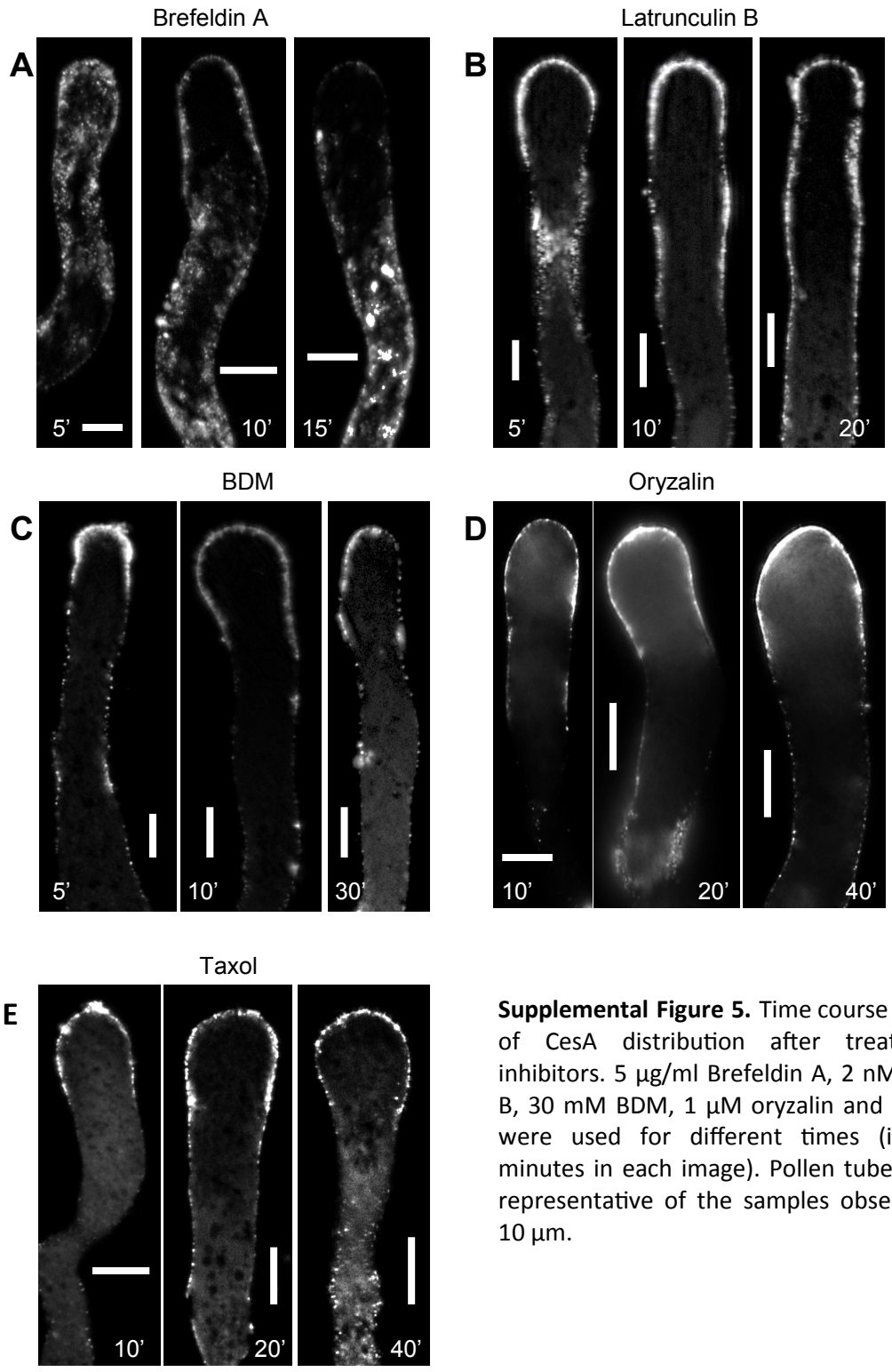
Supplemental Figure 3. Time course experiments of CalS distribution after treatment with inhibitors. 5 µg/ml Brefeldin A, 2 nM Latrunculin B, 30 mM BDM, 1 µM oryzalin and 10 µM taxol were used for different times (indicated as minutes in each image). Pollen tubes shown are representative of the samples observed. Bars = 10 µm.



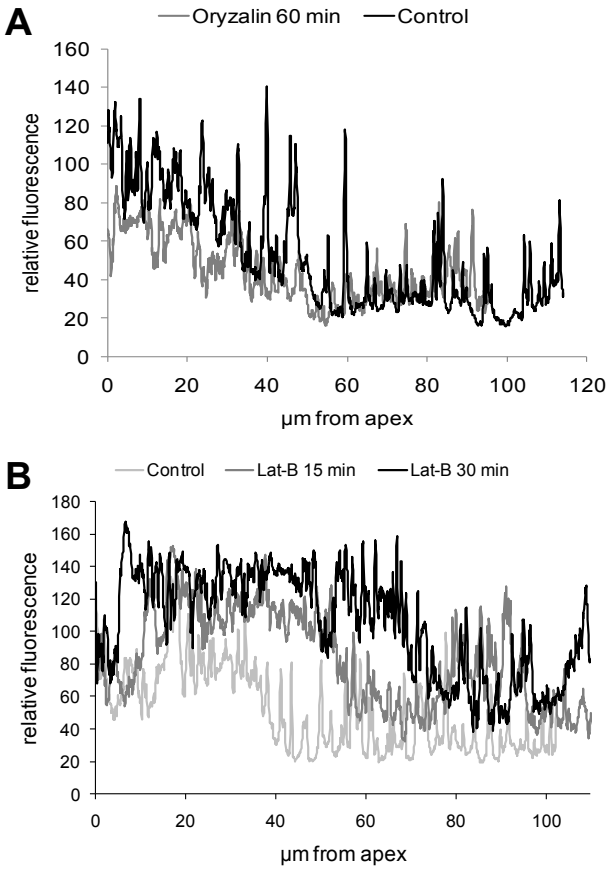
Supplemental figure 4

Distribution of CalS after treatment with different inhibitors.

To determine quantitatively the changes in CalS accumulation, the pollen tube was divided in 10-µm increments along its length and the fluorescence intensity from different treatments was measured in each region. As shown in the line graph, CalS accumulated significantly in the region 80–90 µm behind the apex after BFA treatment and then it dropped down. A second more intense accumulation area was found after treatment with either taxol or oryzalin around 120 µm behind the apex. Values are averages of ten different measures performed on pollen tubes with equivalent length.



Supplemental Figure 5. Time course experiments of CesA distribution after treatment with inhibitors. 5 µg/ml Brefeldin A, 2 nM Latrunculin B, 30 mM BDM, 1 µM oryzalin and 10 µM taxol were used for different times (indicated as minutes in each image). Pollen tubes shown are representative of the samples observed. Bars = 10 µm.

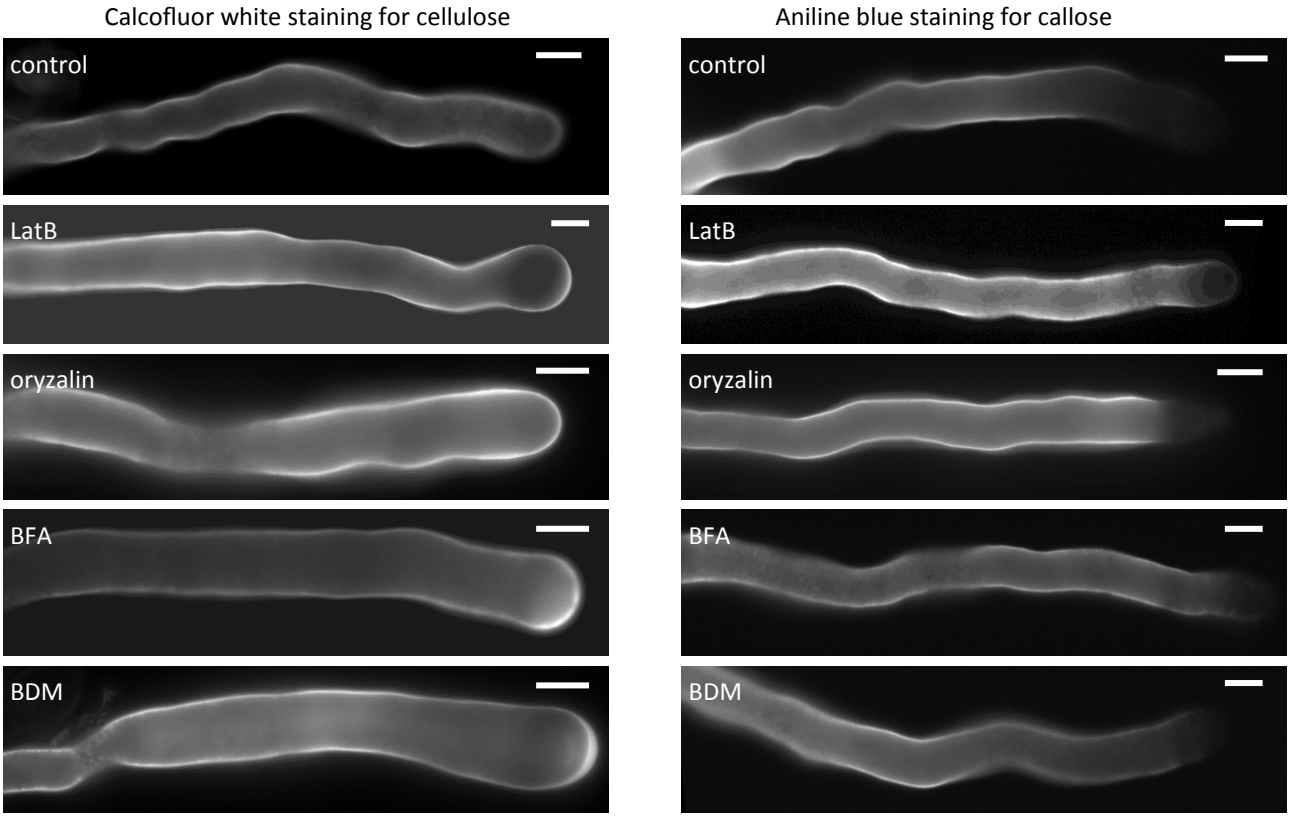


Supplemental figure 6

Fluorescence intensity of CesA measured along the pollen tube border. Values are averages of ten different measures performed on pollen tubes with equivalent length.

(A) Fluorescence intensity measured from the tip in the absence or presence of oryzalin (60-min treatment), revealing the irrelevant effect of the inhibitor.

(B) Fluorescence intensity measured along the pollen tube border from the tip in the absence or presence of LatB (15 and 30 min-treatment, respectively) showing the progressive relocation of CesA proteins toward the tube base.



Supplemental figure 7

Staining of cellulose (left panel) and callose (right panel) with calcofluor white and aniline blue, respectively. Staining was done in untreated (control) pollen tubes and in pollen tubes treated with inhibitors at the concentrations and times indicated in the text. Bars: 10 µm.

Supplemental Video S1 and S2

Effects of BDM on the pollen tube of tobacco. Pollen tubes were treated with 30 mM BDM and were observed soon after the beginning of treatment for 15-30 minutes. Single pollen tubes were recorded using the Zeiss Axio Imager optical microscope, equipped with a 100X objective, under differential interference contrast (DIC) illumination. Video sequences were captured with the MRm AxioCam video camera using AxioVision software; videos were recorded at 1 frame/sec and saved as AVI files (Radius Cinepak codec). For fast visualization of BDM effects, the frame rate of video sequences was set at 30 frames/s in order to obtain a 30X speed. Videos were converted to MOV files (XviD codec).

Video S1: BDM treated tube plus control. Double video sequence showing a control pollen tube (top) and a BDM-treated pollen tube (bottom). Both video sequences have the same duration (15 min, 30 X). In the treated sample, the speed of organelle flow visibly decreases while it remains constant in the control sample. Bar = 10 μm.

Video S2: BDM treated tube. In this case, organelle movement stops after a 20-min treatment (30X).