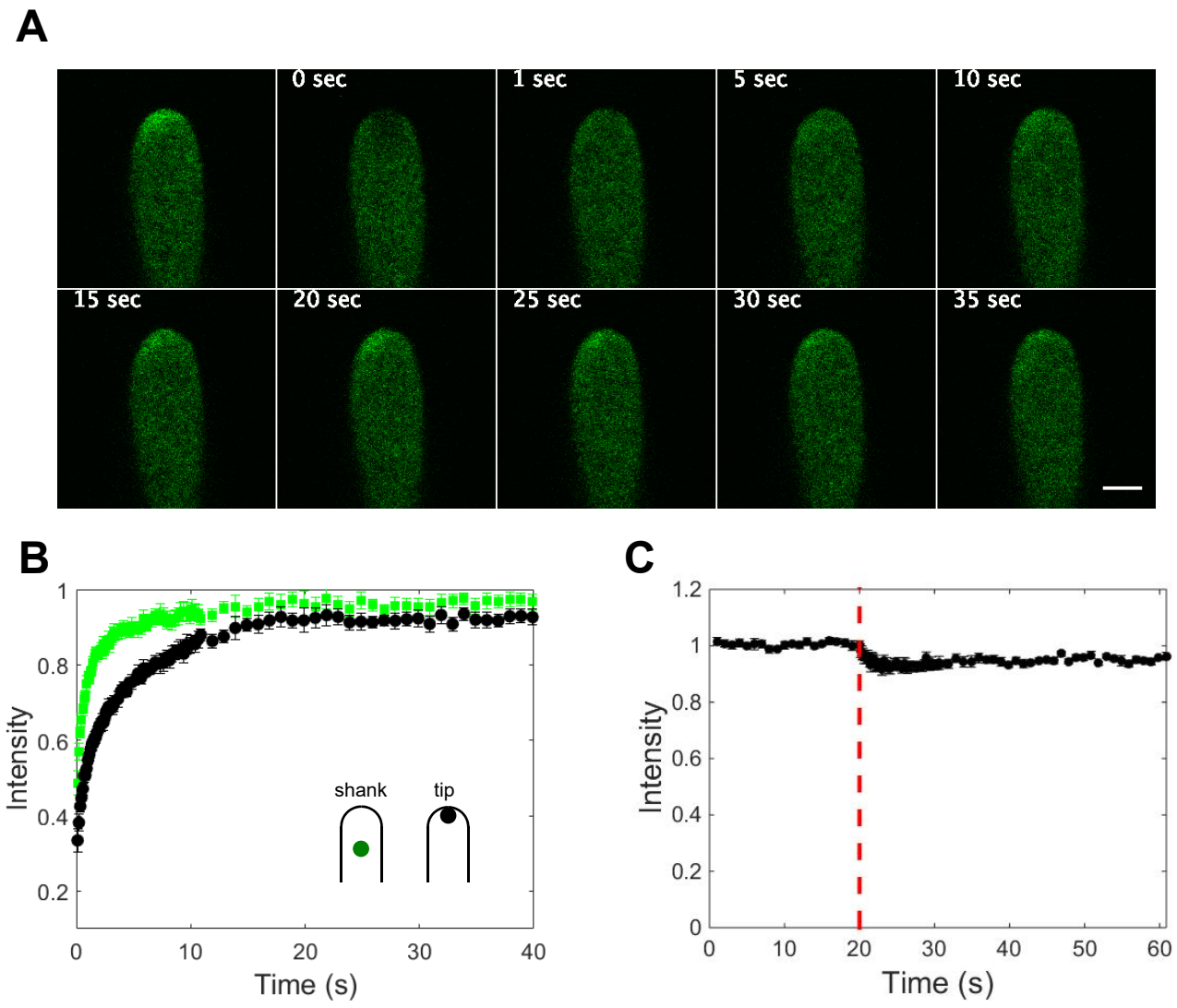



**Figure S1.** Myosin XIa fluorescence during photo bleaching. **A)** Representative images of bleaching and recovery sequence for 3mEGFP-myosin XIa at the tip of the cell. These images were extracted from Sup Movie 1. Scale bar is 5 μm. **B)** Fluorescence recovery of 3mEGFP-myosin XIa at the cell shank (green circles) and the cell tip (black circles). n=7 and 10 cells for the shank and tip, respectively. Fluorescence from tip and shank regions was normalized to the pre-bleach fluorescence of their respective region (error bars indicate standard error of the mean). **C)** Fluorescence intensity of myosin XIa at the cell shank during tip photo bleaching experiments. n=8 cells (error bars indicate standard error of the mean).

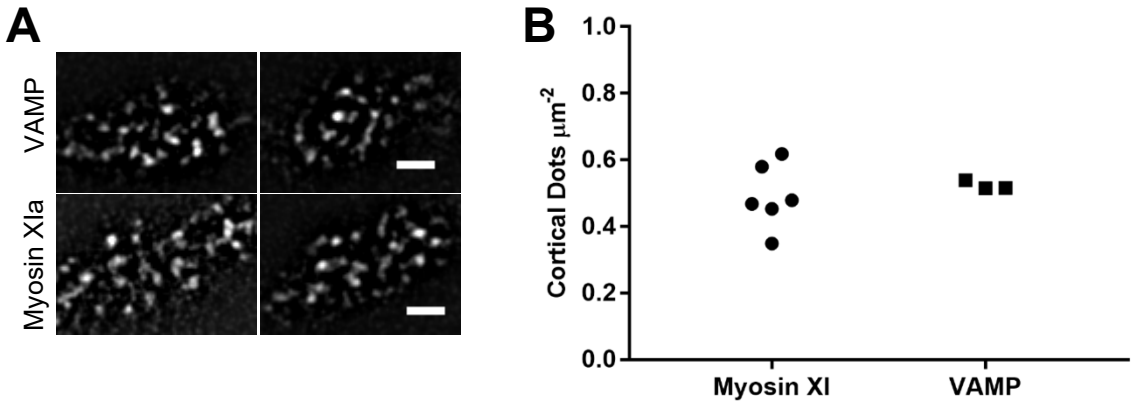



**Figure S2.** VAMP72 and Myosin XIa localize to punctate structures at the cell cortex of moss cells. **A)** Two representative images of cortical localization of 3mEGFP-VAMP72 and 3mEGFP-Myosin XIa in apical caulonemal cells acquired using VAEM. Scale bars are 2 µm. **B)** Density of cortical punctate structures of 3mEGFP-VAMP72 and 3mEGFP-Myosin XIa in apical caulonemal cells. n=6 cells for 3mEGFP-Myosin XIa and n=3 cells for 3mEGFP-VAMP72.

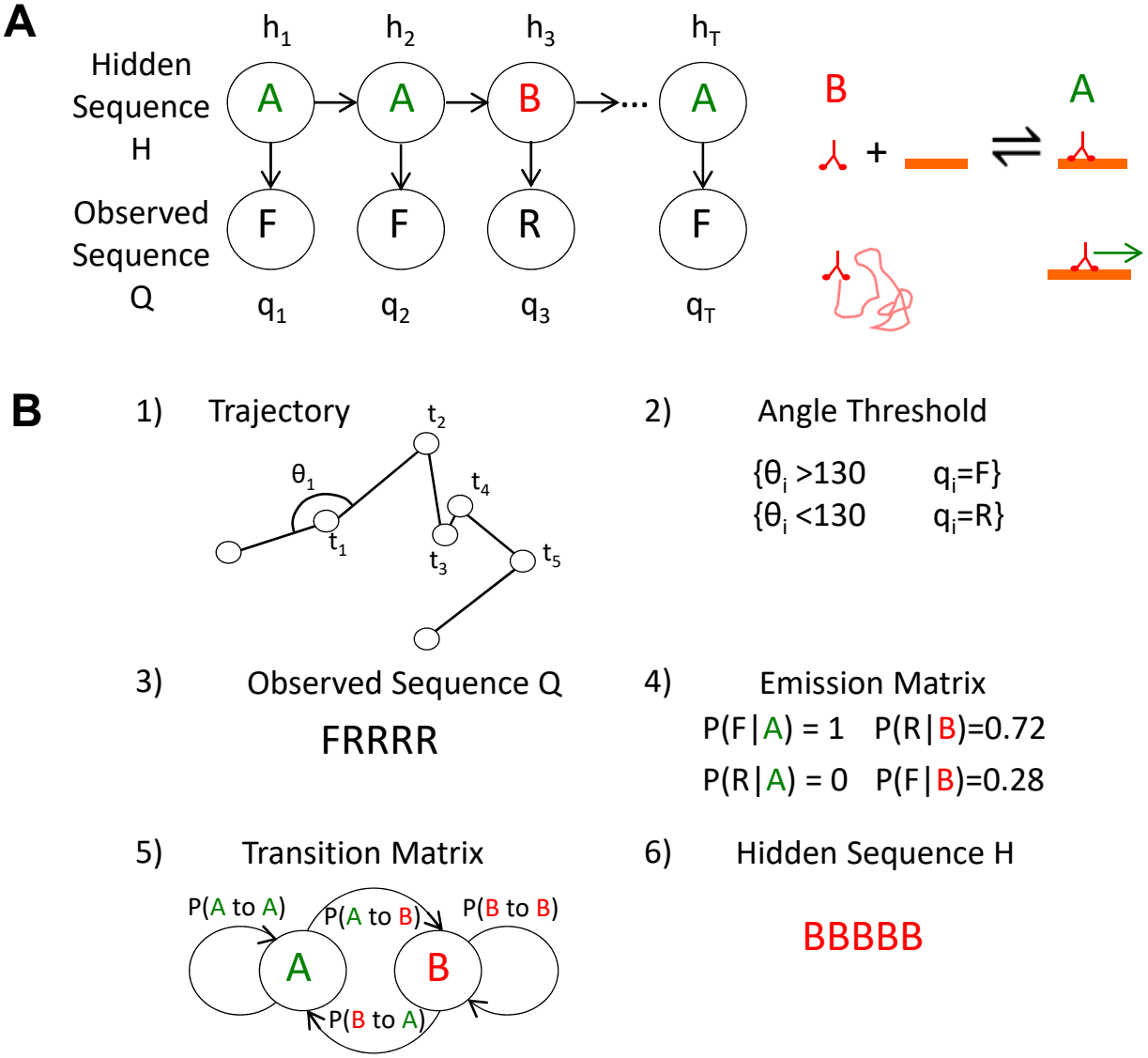



**Figure S3.** Hidden Markov model explained**. A)** Example hidden sequence *H* and the corresponding observed sequence *Q***.** In the hidden sequence green *A*s correspond to the active transport state and red *B*s correspond to the Brownian state. In the observed sequence, black *F*s correspond to forward moves and black *R*s correspond to backward moves. **B)** Example of trajectory conversion from observed sequence *Q* to the hidden sequence *H*. Step 1) indicates how the angle $\theta_i$ was measured across the trajectory. Step 2) shows how that angle was thresholded at 130° to produce forward and backward moves. Step 3) shows the final converted observed sequence *Q*. Step 4) Assume given emission matrix. Step 5) Find transition matrix that maximizes the likelihood of the observed sequence *Q*. Step 6) Find most likely hidden sequence *H*.

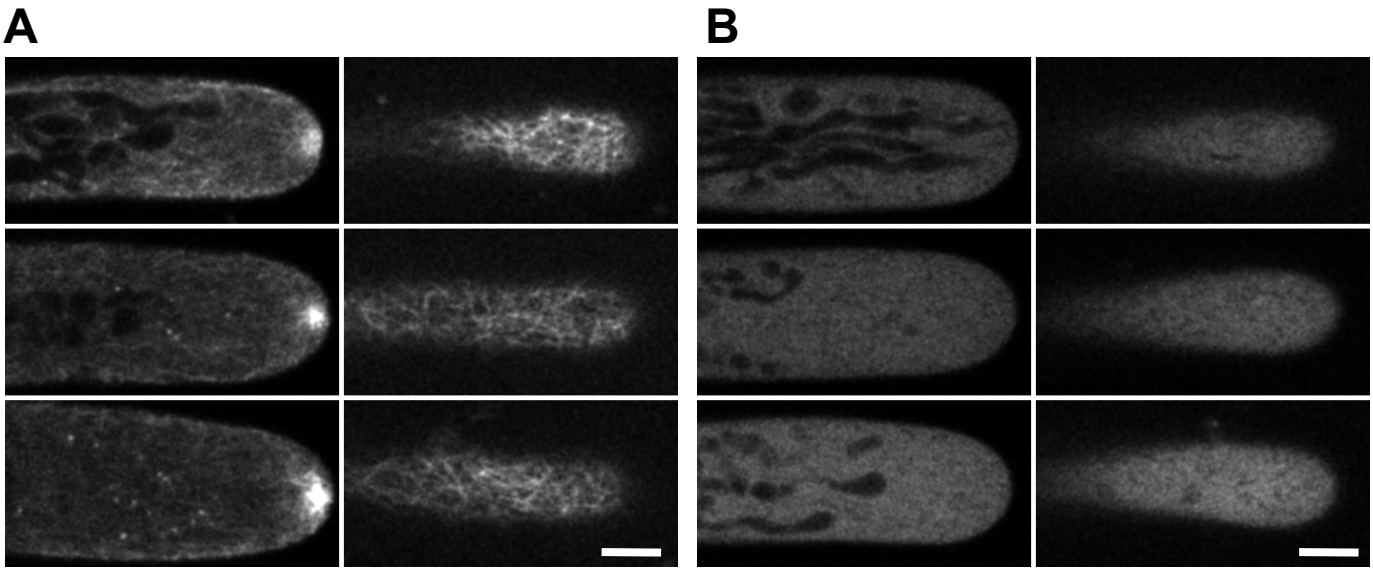



**Figure S4.** Latrunculin B treatment depolymerizes apical and cortical F-actin. **A)** Three representative examples of untreated caulonemata (controls). This cell line expresses lifeact-mCherry, which allows for the visualization of F-actin. Note that apical (left) and cortical (right) F-actin structures are visible. **B)** Three representative examples of caulonemata treated with 25 μM latrunculin B for 10 min. Same cell line as in (A); note that apical and cortical F-actin structures are not detectable. (A and B) Cells were cultured for one week in $PpNO_3$ medium. For treatment and visualization, the cells were placed on an agar pad containing latrunculin B and 20 μl of latrunculin B containing medium was added on top. The cells were covered with a glass coverslip and sealed. Imaging was done with an SP5 (Leica) laser scanning confocal microscope using a 63x N.A. 1.4 lens and a 561 nm excitation laser. Images from the medial and cortical planes were contrast enhanced (0.1% saturated pixels) and Gaussian blur filtered (alpha(radius) = 1.2 pixels) using ImageJ. Scale bar is 5 μm.

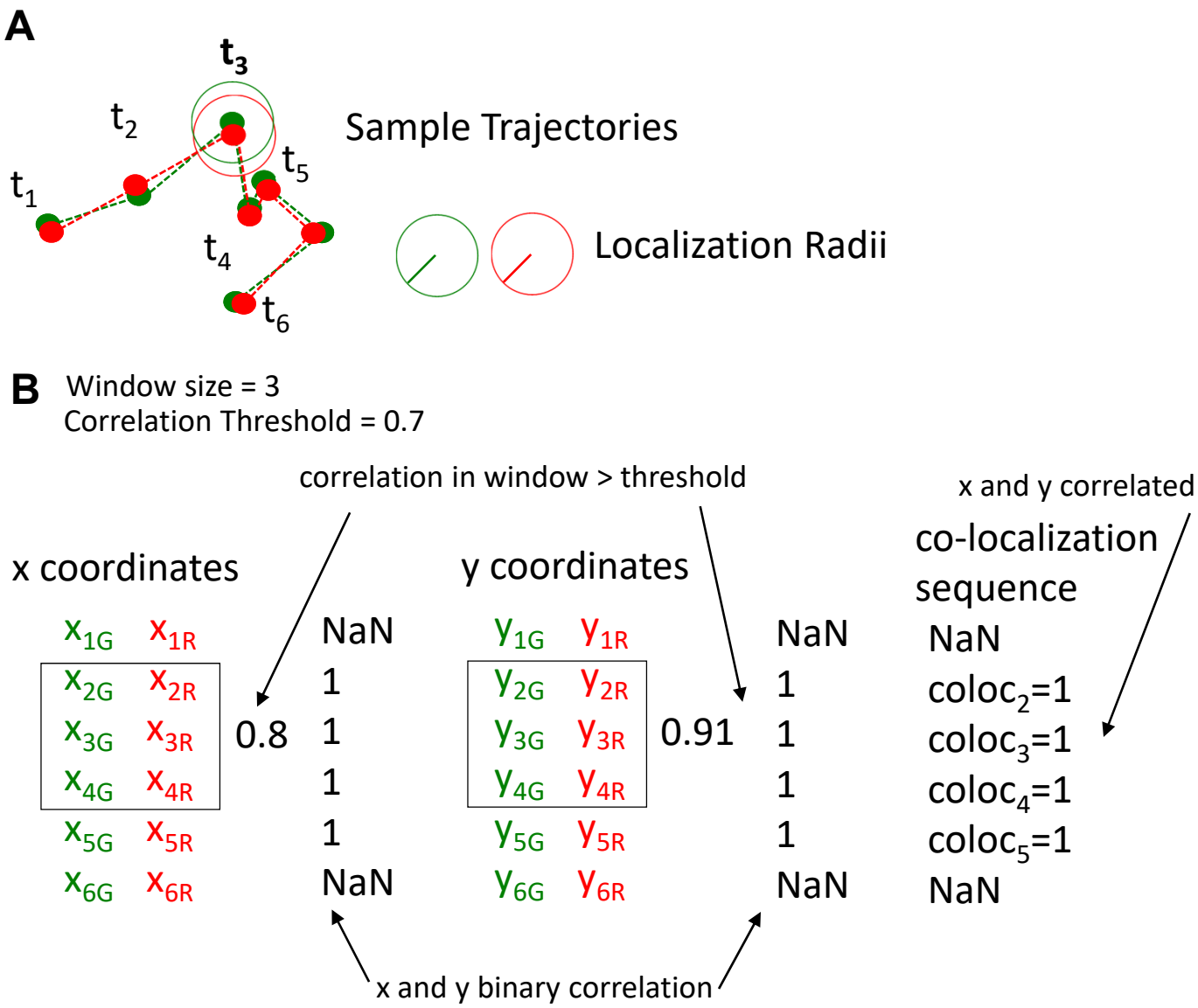



**Figure S5.** Example co-localization analysis. **A)** Sample trajectories from the red and green channels and the specified localization radius for $t_3$. **B)** Correlation analysis for x and y coordinates at $t_3$ for a window size of 3 frames. Coordinate colors indicate channel. In the example the red and green channels exhibit a correlation above the given threshold in both the x and y directions and are classified as 1. Because both directions are correlated, $t_3$ is classified as co-localized. $coloc_2$ and $coloc_6$ are labeled NaN because the window size does not allow for co-localization at these points in the trajectory.

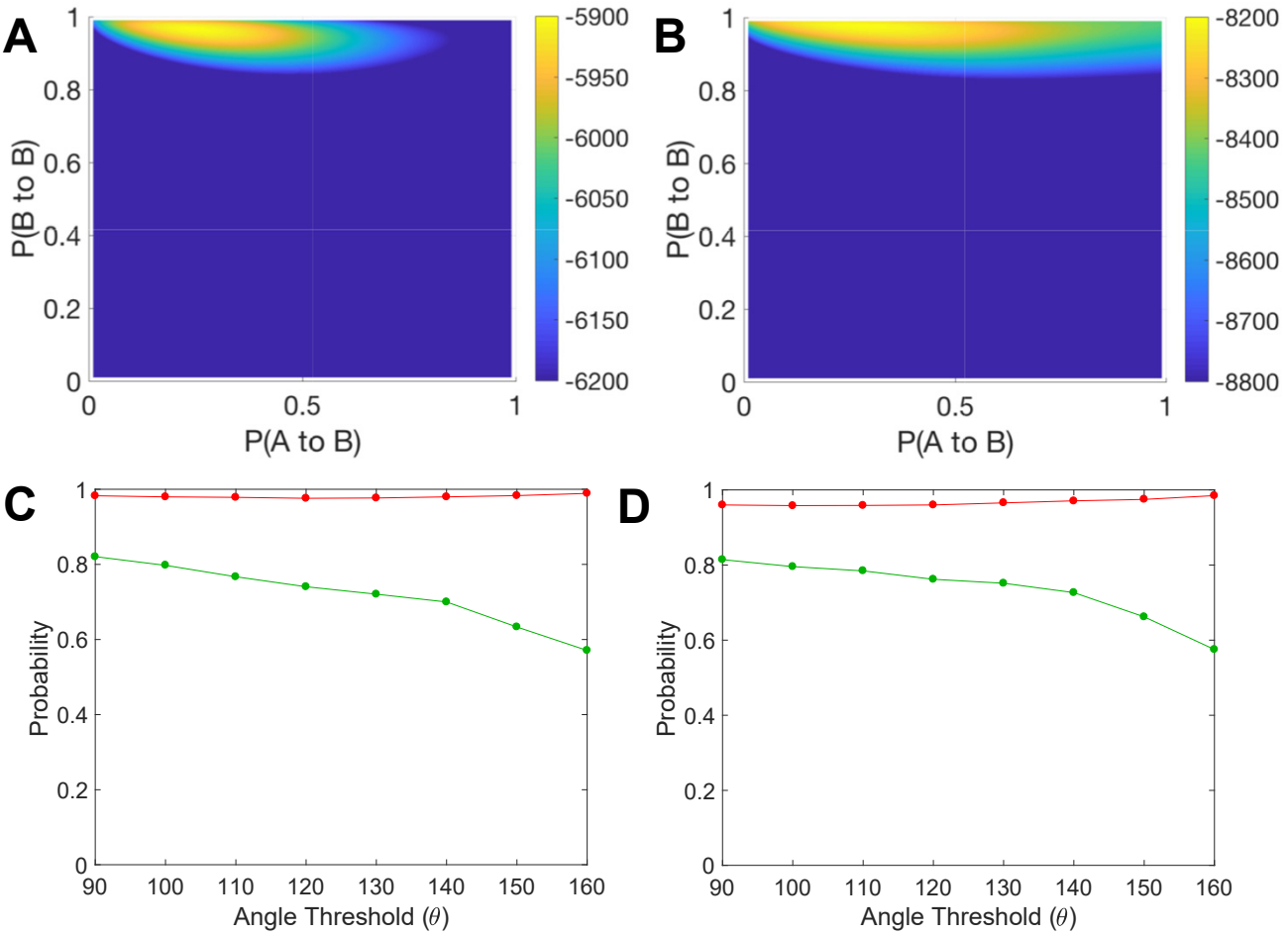
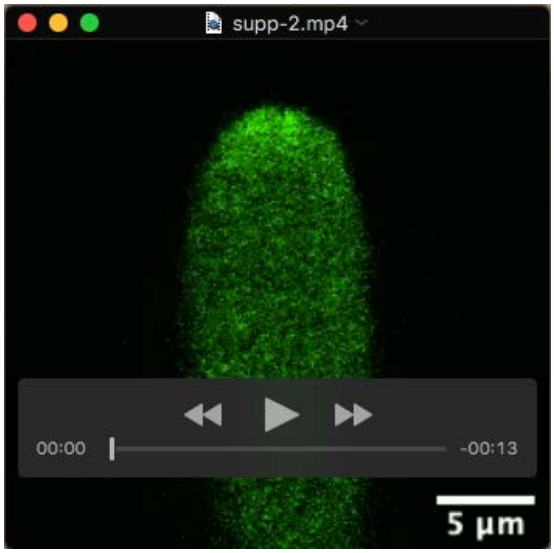
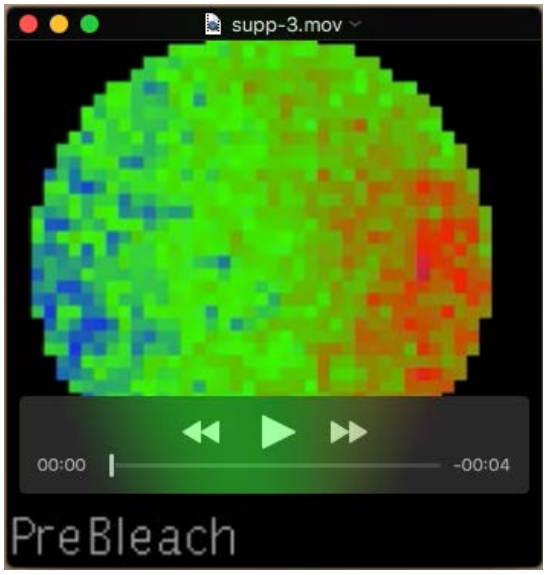



**Figure S6.** HMM sensitivity and convergence analysis. **A and B)** Log-likelihood of all the observed sequences of VAMP72-labeled vesicles (A) and myosin XIa (B) in protoplasts for an array of possible transition matrices. x and y-axes represent $P(h_t = A \mid h_{t-1} = B)$ and $P(h_t = B \mid h_{t-1} = B)$, respectively. Blue to yellow color map indicates log-likely hood where blue indicates less likely observed sequences and yellow indicates more likely sequences. A clear global maximum can be found in both (A) and (B). **C and D)** Hidden Markov Model myosin XIa (C) and VAMP72-labeled vesicle (D) transition probabilities in protoplasts, as a function of the angle threshold. Red lines indicate the Brownian to Brownian transition probability, $P(h_t = B \mid h_{t-1} = B)$. Green lines indicate active to active transition probability, $P(h_t = A \mid h_{t-1} = A)$.

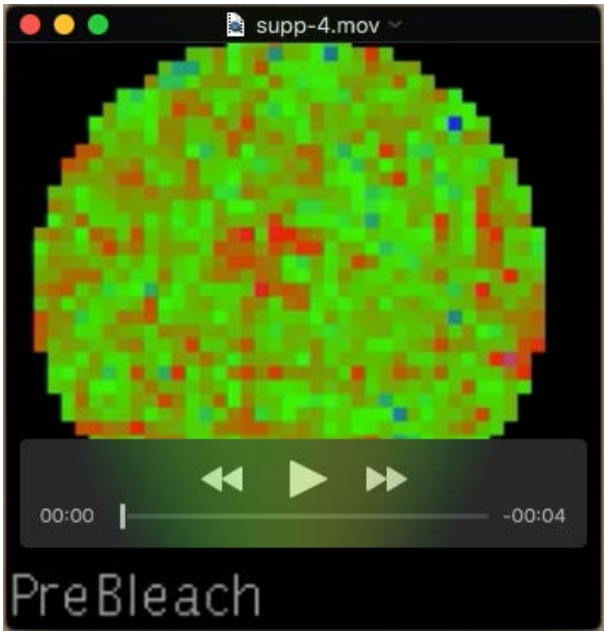
## Supplemental Movies



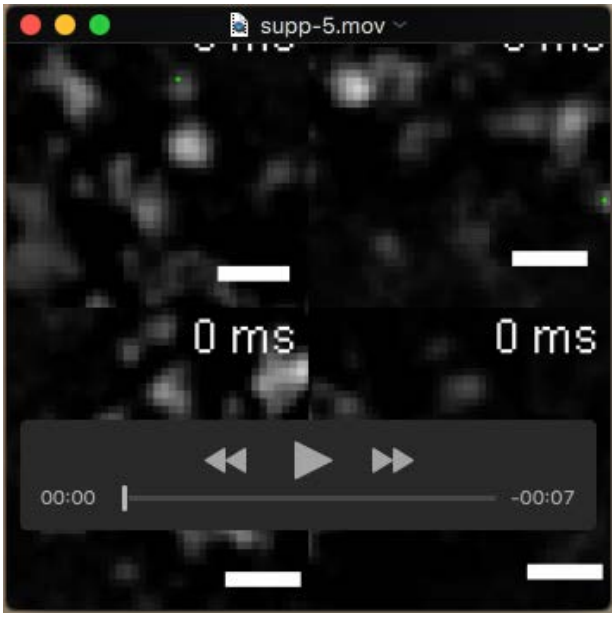

**Movie 1.** Representative movie of the bleaching process and imaging of the whole cell during recovery. Fluorescence bleaching takes place at the most apical region of the cell in a circular area of 4 µm in diameter. Bleaching was conducted on an SP5 confocal microscope (Leica).



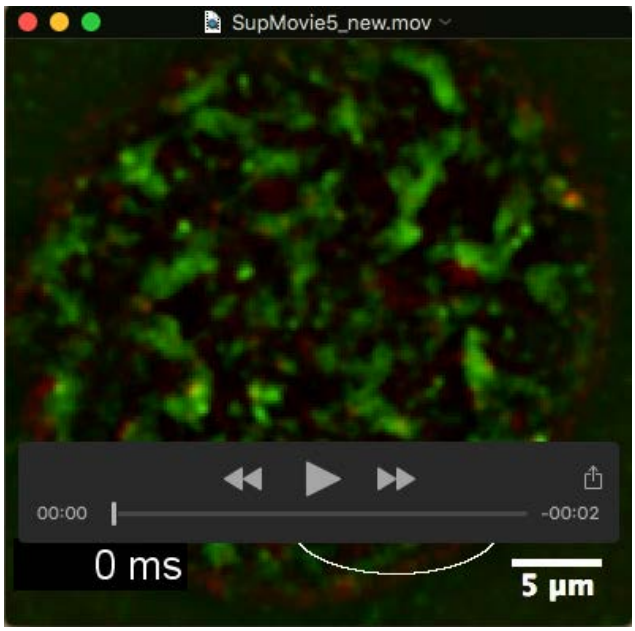

**Movie 2.** Cropped and averaged fluorescence recoveries of myosin XIa at the cell tip. Rainbow lookup table denotes intensity where warm colors are high intensities and cool colors are low intensities. ROI is 4 µm in diameter. n=10.



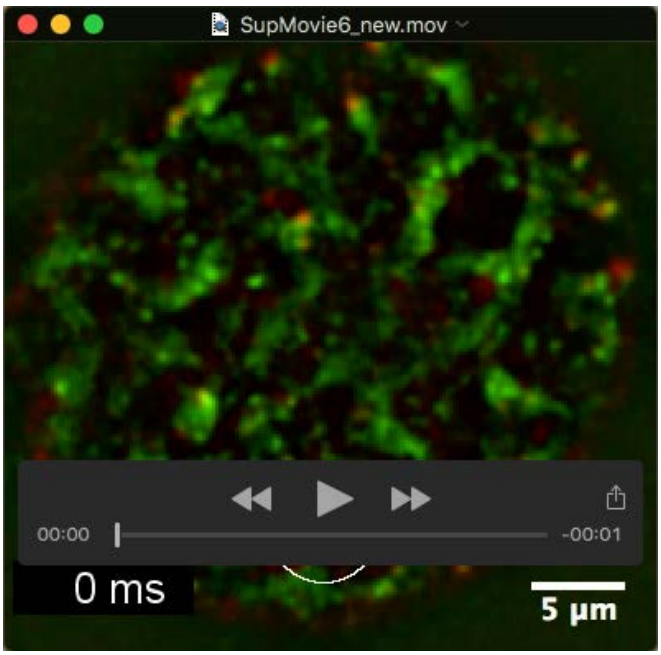

**Movie 3.** Cropped and averaged fluorescence recoveries of myosin XIa in Latrunculin B treated cells at the tip. Rainbow lookup table denotes intensity where warm colors are high intensities and cool colors are low intensities. ROI is 4 µm in diameter. n=8.



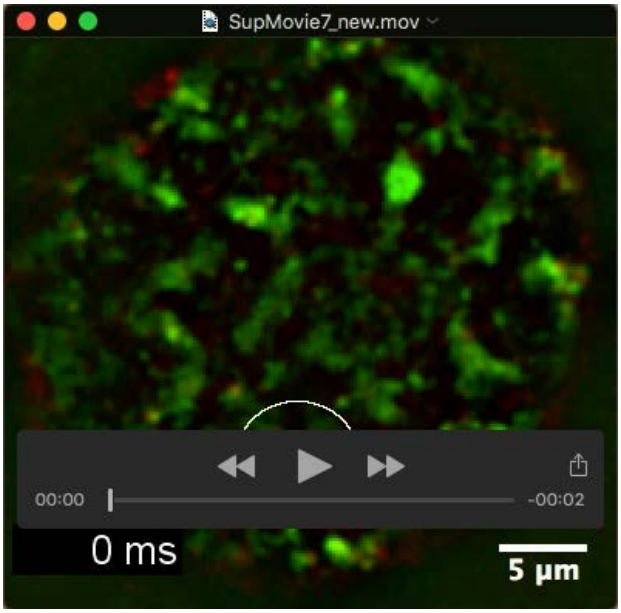

**Movie 4.** Examples of myosin XIa and VAMP72 directed trajectories in caulonemata. Top two sequences are for 3mEGFP-myosin XIa signal, bottom two sequences are for 3mEGFP-VAMP72 vesicles. The green dot indicates the tracked signal and a red line on the last frames show the entire trajectory. Scale bar is 1 µm.



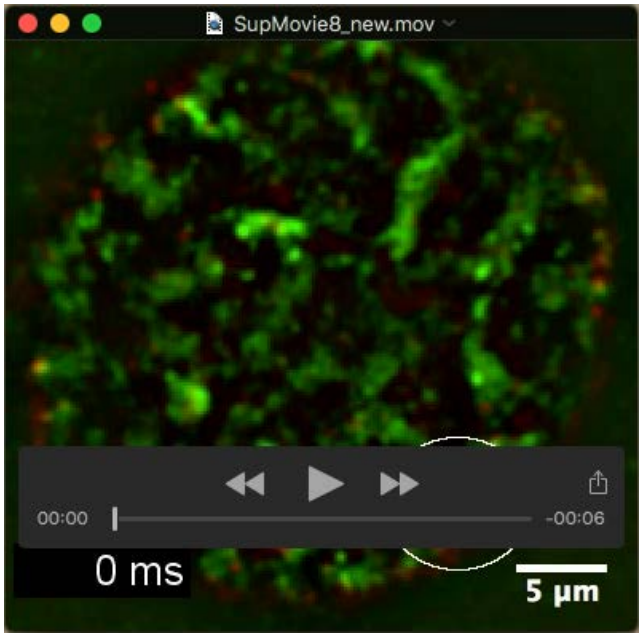

**Movie 5.** Example VAMP72 HMM predicted active trajectory in protoplast with no co-localization. Blue line indicates HMM predicted active trajectory. Red channel in 3mCherry-VAMP72 and green channel is 3mEGFP-myosin XIa.




**Movie 6.** Example myosin XIa HMM predicted active trajectory in a protoplast with no co-localization. Blue line indicates HMM predicted active trajectory. Red channel in 3mCherry-VAMP72 and green channel is 3mEGFP-myosin XIa.




**Movie 7.** Co-localized myosin XIa and VAMP72 HMM predicted active trajectories in protoplast. Blue line indicates HMM predicted active trajectory. Red channel in 3mCherry-VAMP72 and green channel is 3mEGFP-myosin XIa.




**Movie 8.** Co-localized myosin XIa and VAMP72 HMM predicted Brownian trajectories in protoplast. Blue line indicates co-localized HMM predicted Brownian trajectory. Red channel in 3mCherry-VAMP72 and green channel is 3mEGFP-myosin XIa.