Supplemental Materials

Molecular Biology of the Cell

Basu et al.

Supplemental Information

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Force production and turgor pressure during endocytosis in fission yeast.

Supplement includes Supplementary Figures S1-S4, Supplementary Table I and II, and Legend of Supplementary Movies.

Figure S1. Sorbitol effects on patch behavior.

(A) Time-lapse images of a single endocytic patch marked sla1-GFP and crn1-Tomato in wildtype cell in 0.2 M Sorbitol. The onset of inward movement is designated as time = 0. Images are shown at 1 sec intervals. (B) (i) Average lifetimes of sla1-GFP and crn1-tomato after patches begin to move into the cell. Movements were imaged in wildtype (FC2589) cells at the indicated sorbitol concentrations. An endocytic pit marked by both markers begins to ingress (t = 0), and then sla1-GFP disassociates from the patch, followed by disassociation of crn1-tomato. No statistical differences were observed between any conditions. (Gheorghe et al.) Fluorescence intensity of crn1-Tomato in wildtype cells (FC2589) immediately prior to invagination at the indicated sorbitol concentrations (C-D) Behavior of additional endocytic markers. App1-GFP (Abp1-like protein) and fim1-GFP (fimbrin) are actin-binding proteins that can be used to follow the behavior of F-actin at the endocytic site. Graphs showing the (i) average residence time at the cortex, (Gheorghe et al., 2008) average lifetime after patches begin to move into the cell and (iii) fluorescence intensity immediately prior to invagination at the indicated sorbitol concentrations in (C) app1-GFP (FC1082) and (D) fim1-GFP (FC1897) cells. ** represents $p < 0.005$, * represents $p < 0.05$ in comparison with times at 0M sorbitol. Error bars = SD.

Figure S2. Sorbitol does not alter the rate of endocytic pit movement. (A) Graph of average distances traveled by sla1-GFP patches before scission in *gpd1*Δ cells (FC2592) under the indicated sorbitol concentrations. n = 24 and 15 patches for 0 M and 0.05 M sorbitol treatments respectively. Images were acquired every 100 ms. Graph of average distances traveled by sla1-GFP patches in wildtype cells is also shown as a comparison. Error bars = SD.

Figure S3. Sorbitol does not suppress endocytotic defects in cells treated with a high dose of Latrunculin A; Lucifer Yellow dye uptake is actin-independent.
(A) Time lapse images of a single wildtype patch marked with sla1-GFP and crn1-Tomato (FC2589) in a cell treated with 200 μM LatA (high dose). Images are taken at 1s interval. (B) Graph showing behavior of patches after treatment with 200 μM LatA, at indicated sorbitol concentrations. n= 100 patches for each condition. (C) Graph of average distances traveled by sla1-GFP patches in wildtype cells after treatment with 200 μM LatA (blue), 200 μM LatA + 0.2 M sorbitol (red). n = 16 and 23 patches respectively. (D) In budding yeast, it has been shown that sorbitol can suppress the effects of high dose Lat A on endocytosis of the lucifer yellow dye (LY) (Aghamohammadzadeh and Ayscough, 2009). In fission yeast, we found that LY dye internalization occurred in cells treated with the 200-400 μM LatA even without sorbitol addition. Images show LY accumulation in vacuoles in cells treated with 200 μM after 5 min or 400 μM LatA after 10 min. (E) Uptake of the membrane dye FM4-64 is actin dependent. Wildtype cells were treated with or without Latrunculin A for 10 min, incubated in 20 mM FM4-64 for 1 min at room temperature, washed with YE5S and imaged after 10 min.

Figure S4. Estimated forces required for membrane ingression against turgor pressure.
(A) Schematic of an endocytic pit, in which ingression of the base of the pit is opposed by turgor pressure. (B) Graph showing estimated magnitude of force of 1 MPa turgor pressure may exert on the membrane as a function of the radius of endocytic pit as schematized in A, using the relationship of Force = Pressure X Surface Area. The surface area used was $2\pi r^2$ (1/2 surface area of sphere), the area at the rounded base of the pit that includes the lipid bilayer but not intracellular proteins such as actin.

Figure S5. Semi-automated sub-pixel resolution tracking of endocytic patches using MatLab. (A) Image showing selection of an endocytic patch (i) and tracking of its position over time (blue) from the start position (magenta) to the end position (cyan) (Gheorghe et al., 2008)(iii) shows a plot of the patch position over time. Images were acquired in binning 2. Scale bar = 2 μm. (B) (i) Measurement of tracking precision by imaging immobilized fluorescent beads. 0.5 μl of 100 nm beads in water were dried on glass slide. 1 μl water was added before

placing coverslip. Images were acquired with a 488 nm laser (100 ms exposure, 200 camera gain, 300 frames continuous, 1-Z plane). Images were acquired using binning 2 (256 x 256 pixels)(top) or binning 1(512 x 512 pixels)(bottom). Distribution of XY positions of the immobilized bead obtained from sub-pixel resolution tracking shown as a 2D graph (Gheorghe et al., 2008) and histogram (iii). SD = standard deviation. (C) Measurement of tracking precision of a patch. Image showing selection of a single stationary endocytic patch (boxed) in a sla1-GFP cell treated with 2 μM LatA (i) and distribution of XY positions of the patch obtained from sub-pixel resolution tracking shown as a 2D graph (Gheorghe et al.) and histogram (iii). Color code as in B. Images were acquired in binning 1. These results suggest that the position of the patches in cells can be measured using these methods with about 5-10 nm precision. Scale bar = 2 μm.

Supplementary Table 1. List of strains used in this study.

Strain	Genotype	Source
FC2589	*h^+ sla1-GFP:KanMX6; crn1-Tomato-dimer:NatMX4 ade6-M216 leu1-32 ura4-D18*	This study
FC2592	*gpd1::ura4+; sla1-GFP: KanMX6; crn1-Tomato-dimer:NatMX4 ade6-M216 leu1-32 ura4-D18*	This study
FC2587	*wsp1::ura4+; sla1-GFP: KanMX6; crn1-Tomato-dimer:NatMX4 ade6-M216 leu1-32 ura4-D18*	This study
FC2659	*myo1::KanMX6; sla1-GFP: KanMX6; crn1-Tomato-dimer:NatMX4 ade6-M216 leu1-32 ura4-D18*	This study
FC2591	*fim1::KanMX6; sla1-GFP: KanMX6; crn1-Tomato-dimer:NatMX4 ade6-M216 leu1-32 ura4-D18*	This study
FC2590	*end4::ura; sla1-GFP: KanMX6; crn1-Tomato-dimer:NatMX4 ade6-M216 leu1-32 ura4-D18*	This study
FC2660	*arp2-1; sla1-GFP: KanMX6; crn1-Tomato-dimer:NatMX4 ade6-M216 leu1-32 ura4-D18*	This study
FC1082	*app1-GFP ade6-M216 leu1-32 ura4-D18*	Lab collection
FC1897	*fim1-mEGFP:KanMX6 ade6-M216 leu1-32 ura4-D18*	J.Q. Wu

Supplementary Table II. Sorbitol causes significant effects on patch dynamics. Student's t-test of comparisons of sla1 residence times at the cortex in the presence of various concentrations of sorbitol. ** represents $p < 0.005$, * represents $p < 0.05$, NS (non-significant) represents $p > 0.05$.

Sorbitol concentration	**0 M**	**0.0125 M**	**0.025 M**	**0.05 M**	**0.1 M**	**0.2 M**
0 M		**	**	**	**	**
0.0125 M			NS	**	*	*
0.025 M				*	NS	NS
0.05 M					NS	NS
0.1 M						NS

Movie legend

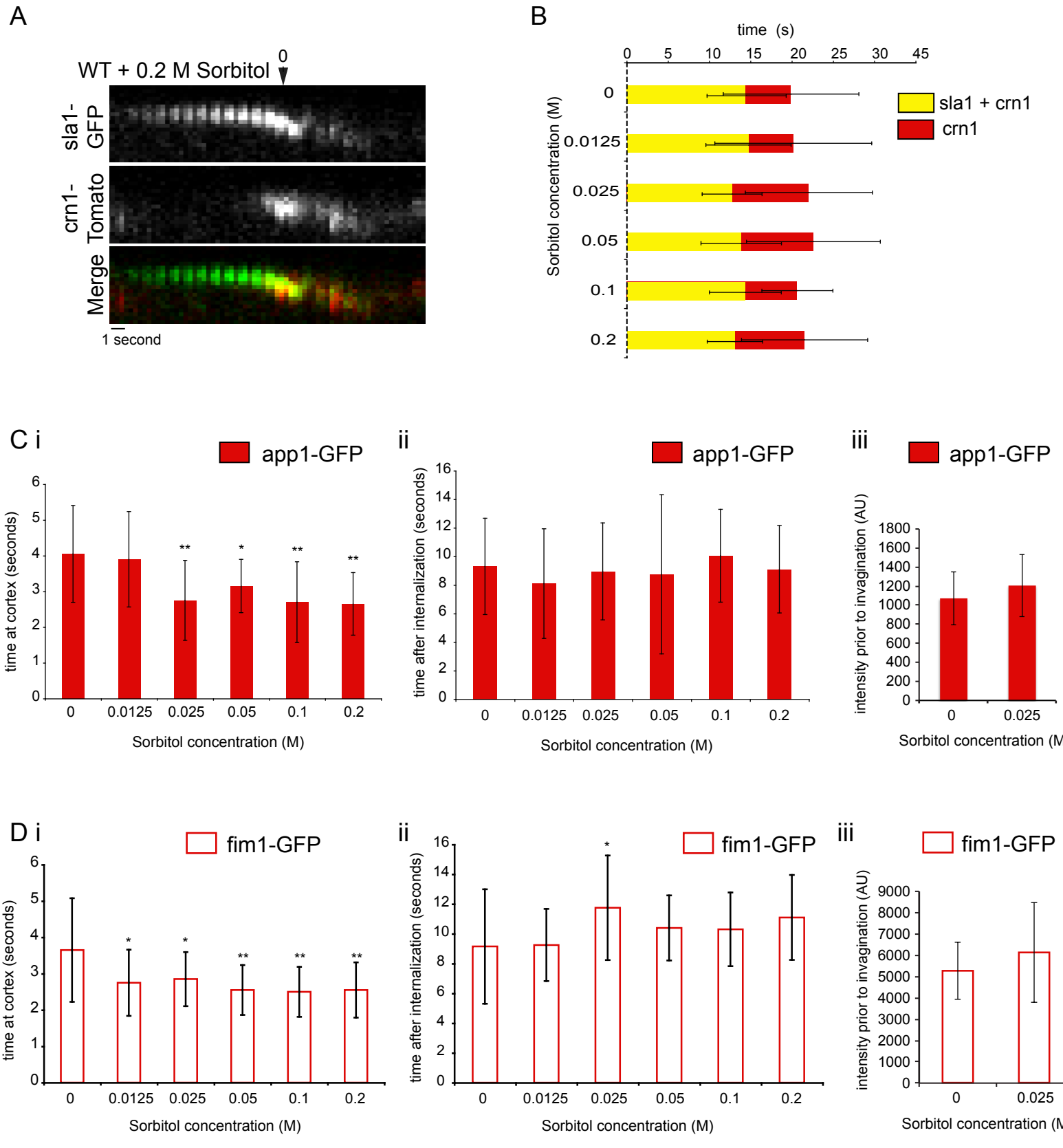
Supplemental Movie 1. Wildtype cells expressing endocytic patch markers sla1-GFP (adaptor protein, green) and crn1-Tomato (coronin; red) (FC2589). Time-lapse images of a medial confocal slice acquired at 1s intervals. Frame rate: 5 frames per second.

Supplemental Movie 2. Wildtype cells treated with 0.2M sorbitol expressing endocytic patch markers sla1-GFP (adaptor protein, green) and crn1-Tomato (coronin; red) (FC2589). Time lapse images of a medial confocal slice acquired at 1s intervals. Frame rate: 5 frames per second.

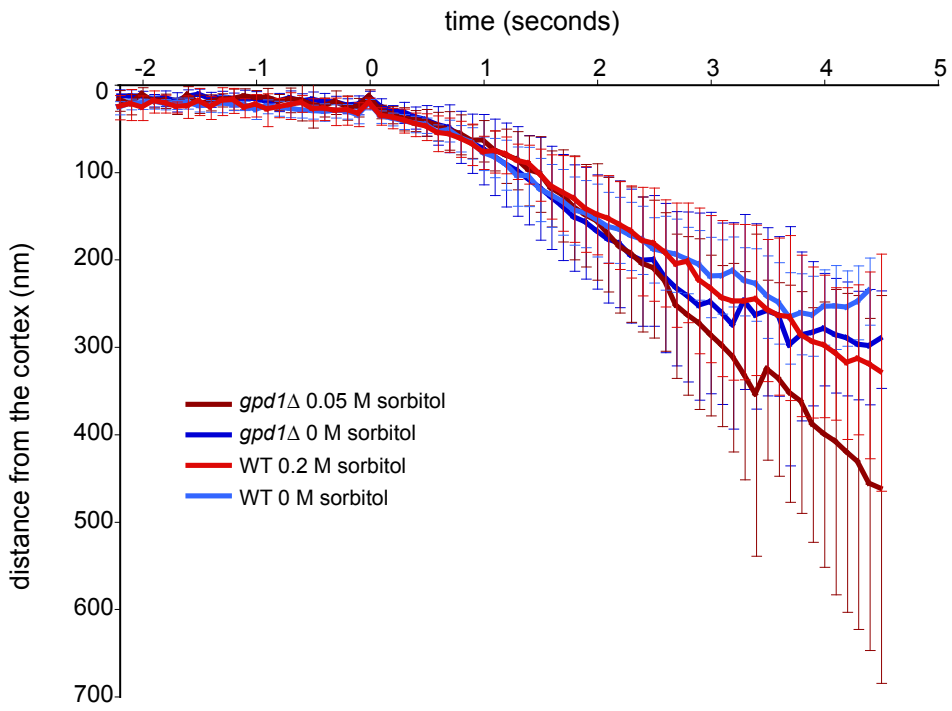
Supplementary references

Aghamohammadzadeh, S., and Ayscough, K.R. 2009. Differential requirements for actin during yeast and mammalian endocytosis. Nat Cell Biol *11*, 1039-1042.

Gheorghe, D.M., S. Aghamohammadzadeh, R. Smaczynska-de, II, E.G. Allwood, S.J. Winder, and K.R. Ayscough. 2008. Interactions between the yeast SM22 homologue Scp1 and actin demonstrate the importance of actin bundling in endocytosis. *J Biol Chem*. 283:15037-15046.



Supplementary Figure S1



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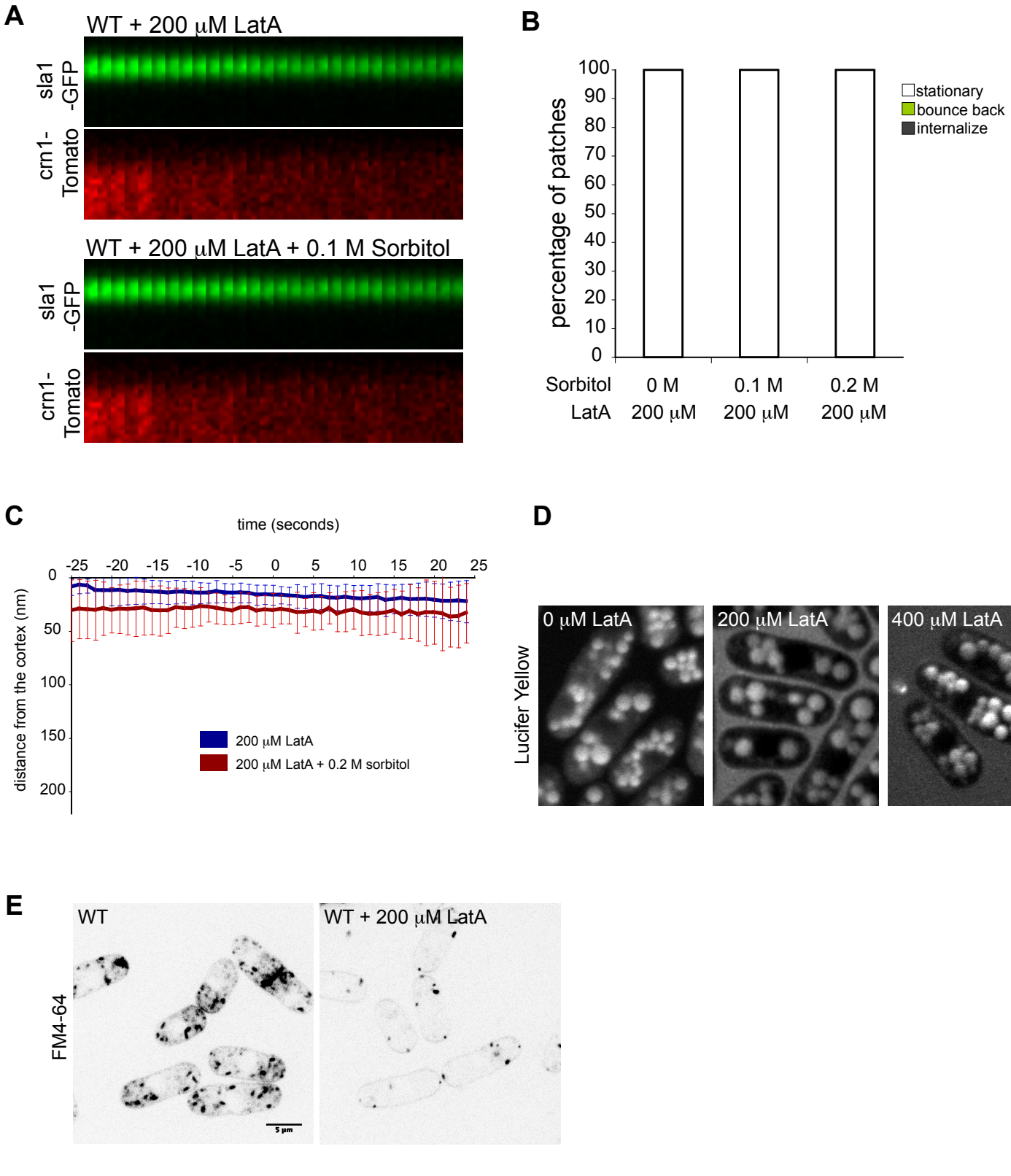

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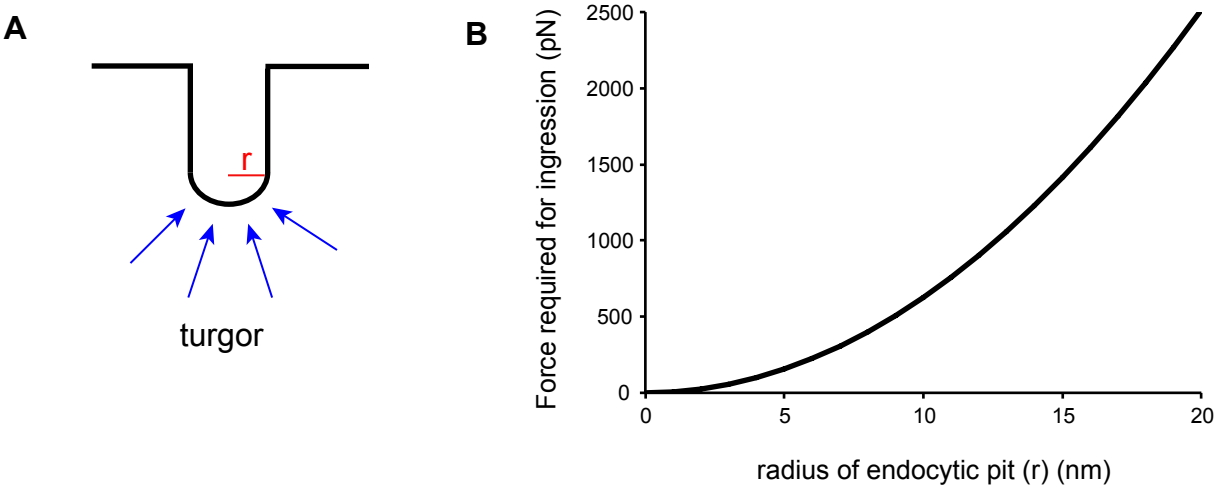
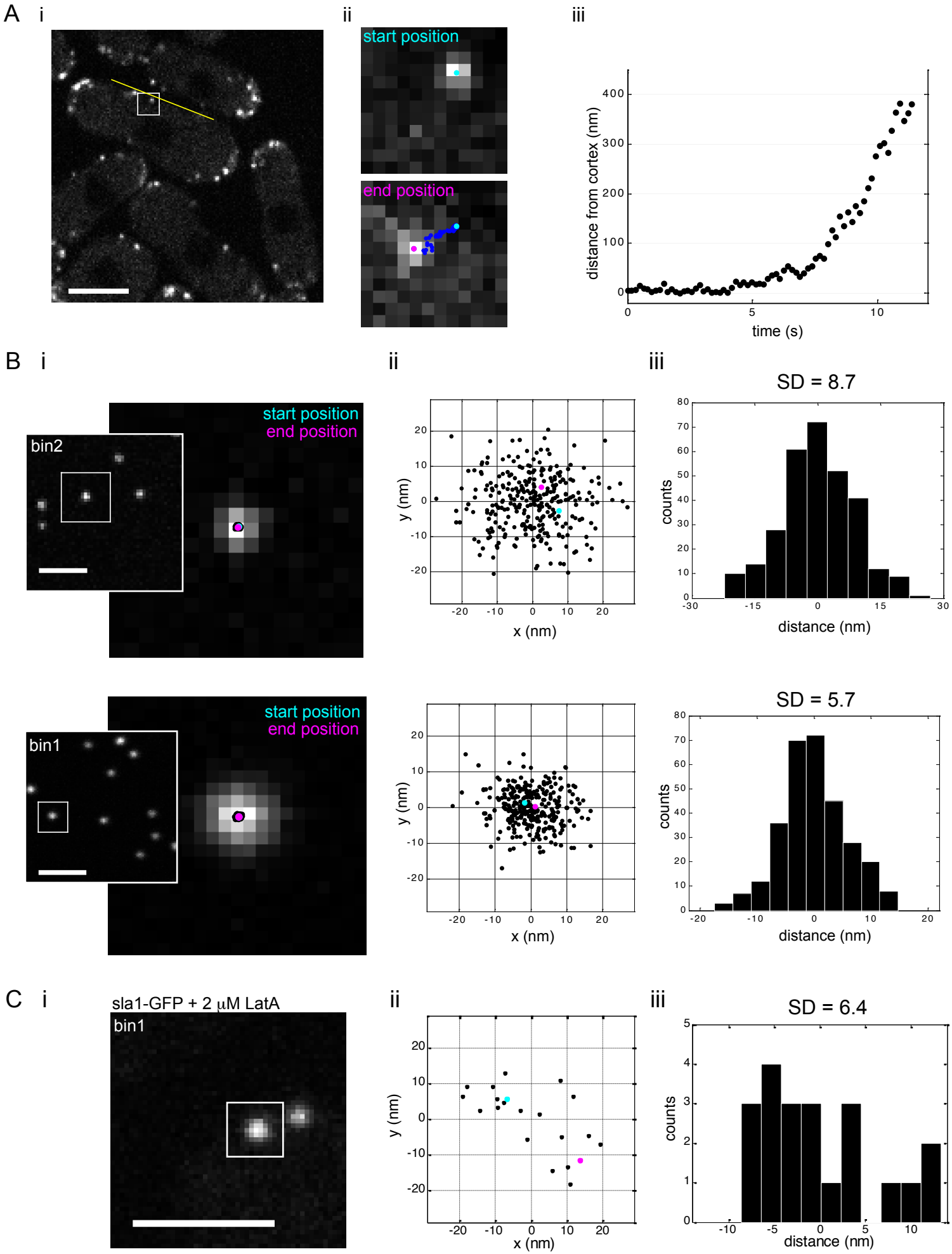


Supplementary Figure S4



Supplementary Figure S5