

Supplemental Materials

Molecular Biology of the Cell

Karlsson et al.

Supplemental Figure S1. Diagram showing the strategy designed to amplify the cassettes used for the split marker endogenous tagging. Cassette containing *sgfp* and *hph* was obtained using plasmid pGFP::hph::loxP (Honda and Selker, 2009; accession number FJ457011) as template, and primers 10xGlyF and loxPR. ORF and 3'UTR sequences (about 1Kb each) were amplified from *N. crassa* genomic DNA using primers xGlyF-xGlyR, and xLoxF-xLoxR, respectively. Left and right arms used for transformation were obtained using as templates the amplicons resulting from the first round of PCR and oligonucleotides xGlyF-hphSMR and hphSMF-xLoxR, respectively. See Table 4 for details on oligonucleotides sequences.

Supplemental Figure S2. Fluorescence intensity profiles along transects running from the cytoplasm, the Spitzenkörper and across the PM of *N. crassa* hyphae (top right boxes in each panel) expressing SEC-6-GFP (A) or EXO-70-GFP (B) and stained with FM4-64.

Supplementary Figure S3. Co-expression of fluorescently tagged variants of SEC-6 and EXO-70 at tips of *N. crassa* hyphae. Note lack of co-localization of SEC-6-GFP and EXO-70-mChFP (third row), which can be seen surrounding two small Spitzenkörper, a typical feature when the hypha is about to branch apically. Note also lack of co-localization of SEC-6-mChFP and EXO-70-GFP (fourth row). Scale bar = 10 μ m.

Supplementary Figure S4. The exocyst components were observed at the apices of germlings that had reached a certain length after 6 - 9 hrs of germination. Exocyst components SEC-3, -5, -6, -8, EXO-70 and EXO-84 tagged with GFP localized as crescents at apices of germlings of *N. crassa*. SEC-3-GFP ($80 \pm 10 \mu$ m; n=12), SEC-5-GFP ($75 \pm 31 \mu$ m; n=16), SEC-6-GFP ($112 \pm 58 \mu$ m; n=20), SEC-8-GFP ($\pm \mu$ m; n=), EXO-70-GFP ($80 \pm 39 \mu$ m; n=36) and EXO-84-GFP ($\pm \mu$ m; n=). Scale bars = 10 μ m.

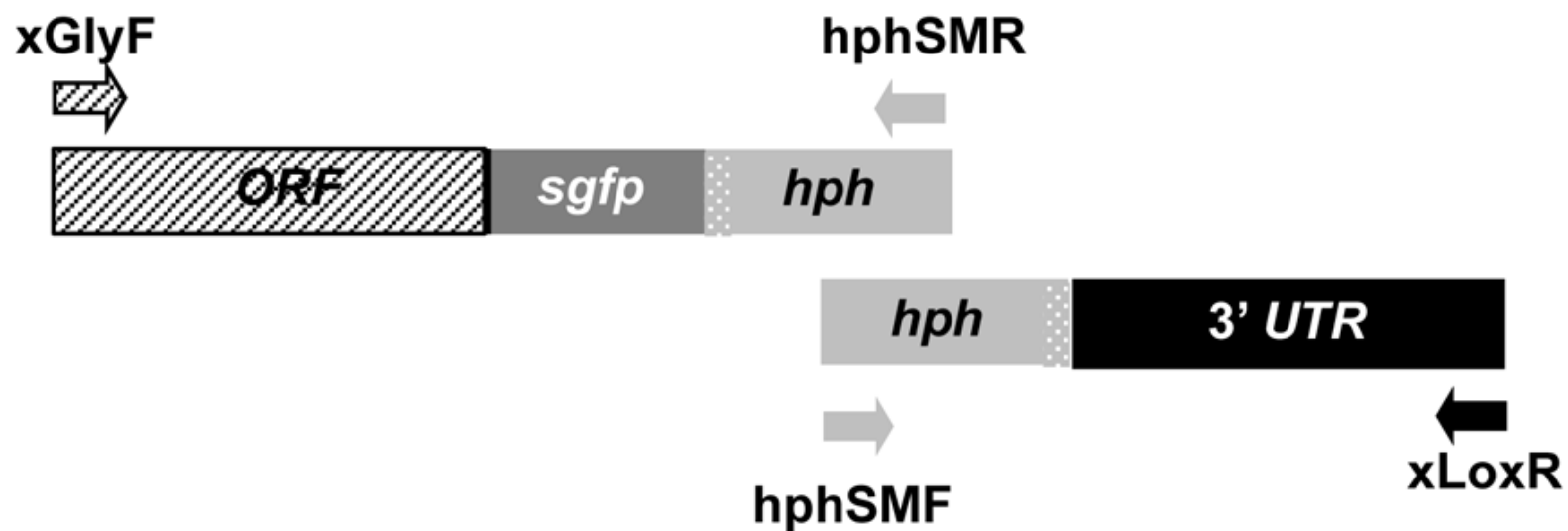
Supplemental Figure S5. Fluorescence intensity profiles along transects running from the cytoplasm, the Spitzenkörper and across the PM of *N. crassa* hyphae (top right boxes in each panel) expressing SEC-6-GFP (A) or EXO-70-GFP (B) and co-expressing CHS-1-mChFP or GS-1- mChFP or stained with FM4-64.

Supplemental Figure S6. Actin cytoskeleton is disturbed in a *N. crassa* *sec-5*⁽⁷⁻⁹⁾ mutant strain. Actin, visualized with Lifeact-GFP (A), accumulates at the Spitzenkörper and the subapical collar in wild type cells (left). In a *sec-5*⁽⁷⁻⁹⁾ mutant (center) actin is no longer at the Spitzenkörper, there is no apparent subapical collar and more actin cables appear. These cables disassembled when latrunculin A was applied (right); Growth was severely impeded in the *sec-5*⁽⁷⁻⁹⁾ mutant and the *sec-5*⁽⁷⁻⁹⁾ mutant expressing Lifeact-GFP (B). Scale bars = 10 µm.

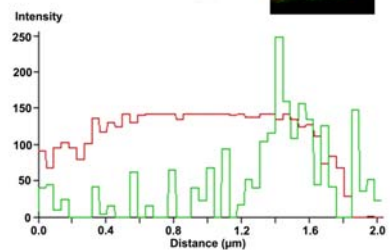
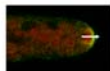
Supplemental Movie 1. Hypha of *N. crassa* co-expressing SEC-6-GFP and CHS-1-mChFP imaged by LSCM. Note the lack of co-localization of the two tags. SEC-6-GFP localizes at the apical plasma membrane, whereas CHS-1-mChFP is found at the core of the Spitzenkörper.

Supplemental Movie 2. Hypha of *N. crassa* expressing SEC-6-GFP imaged by TIRFM.

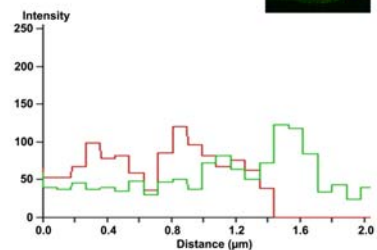
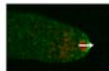
Supplemental Movie 3. Animated three-dimensional plot of the fluorescence intensities of the pixels reaching the plasma membrane over time of 100 frames of the time series in Supplemental Movie 2.



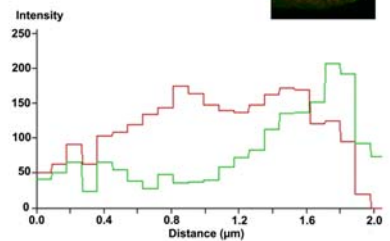
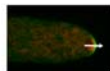
SEC-3-GFP/FM4-64



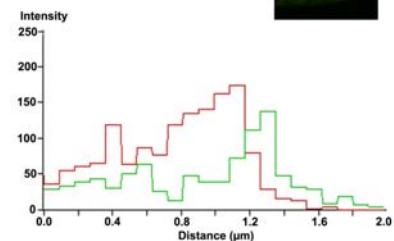
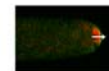
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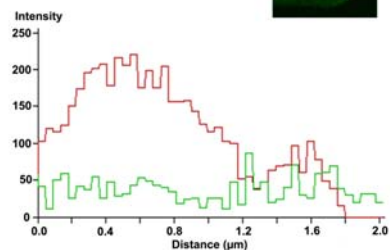
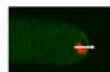
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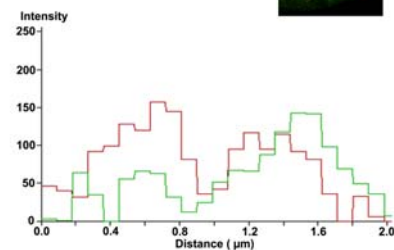
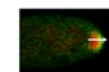
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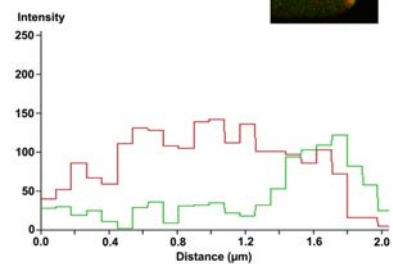
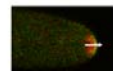
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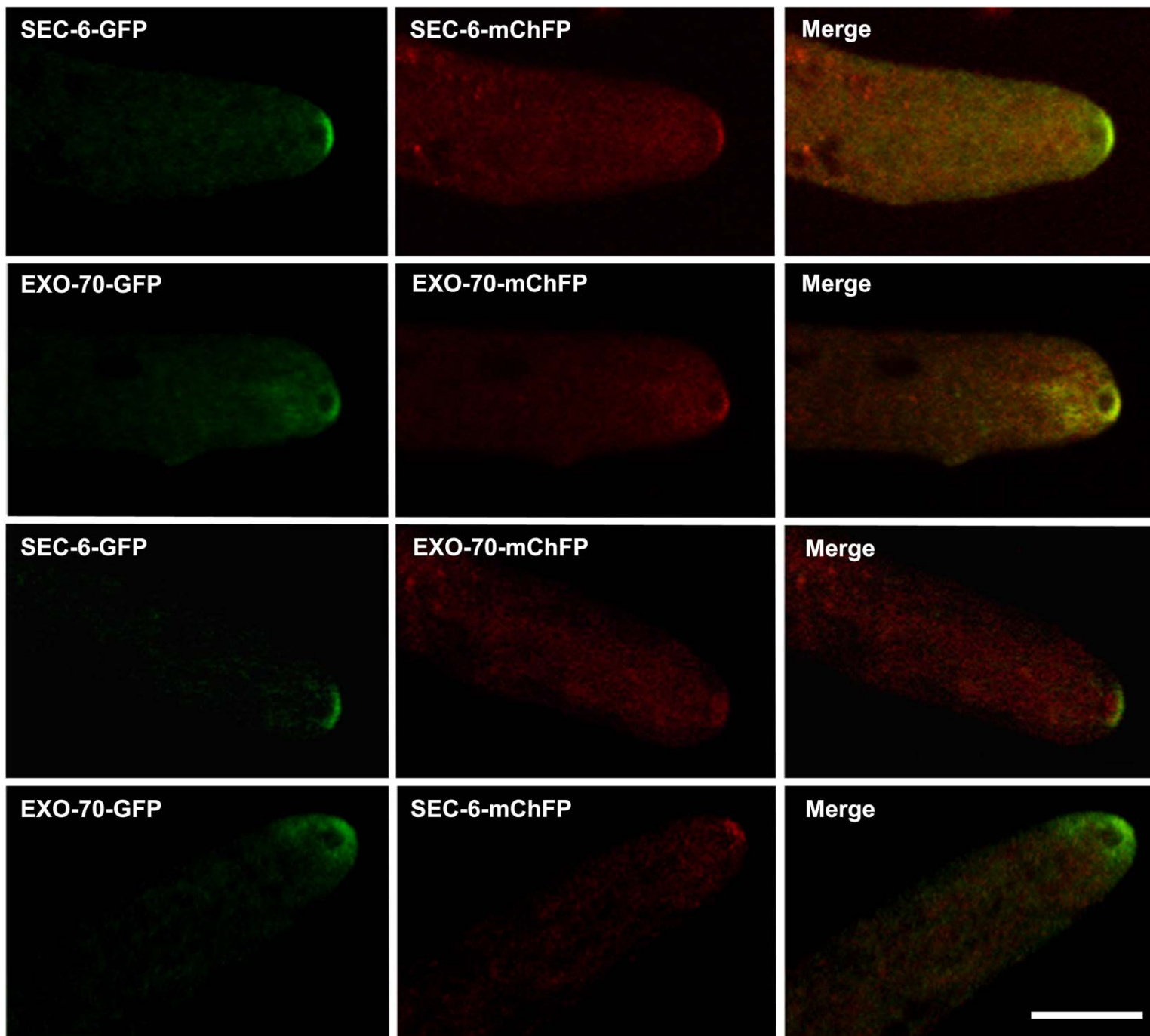


EXO-70-GFP/FM4-64



EXO-84-GFP/FM4-64

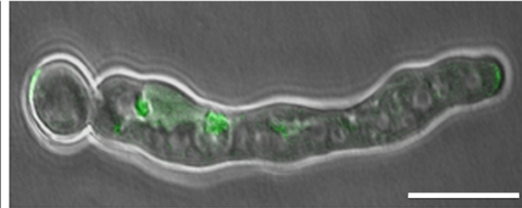
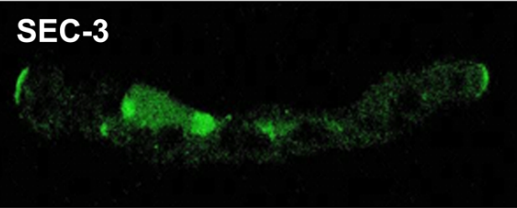




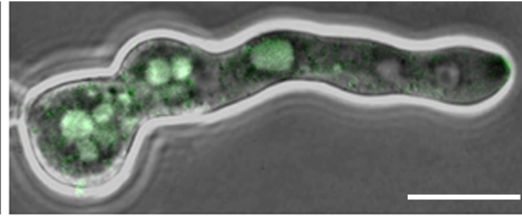
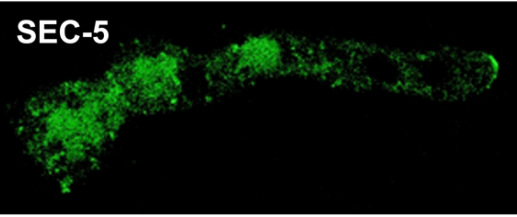
GFP

Merge

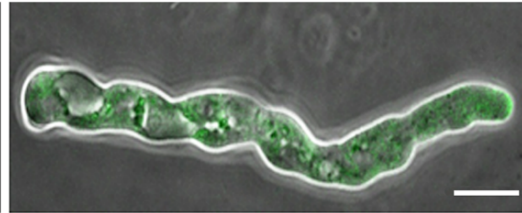
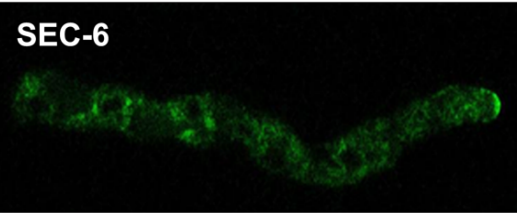
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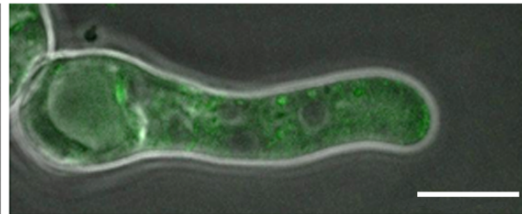
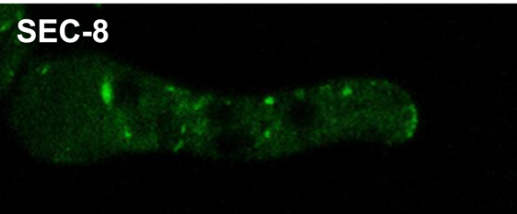
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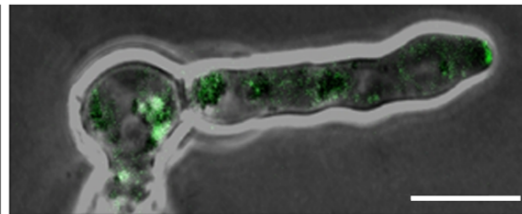
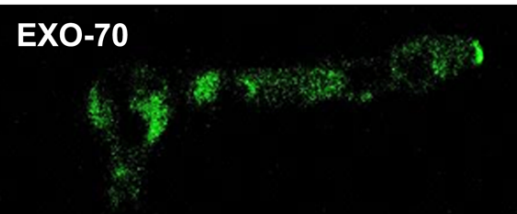
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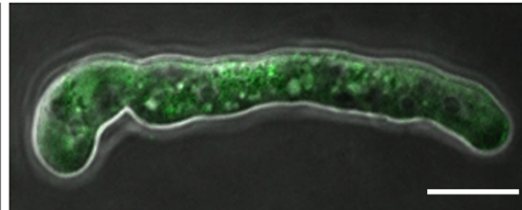
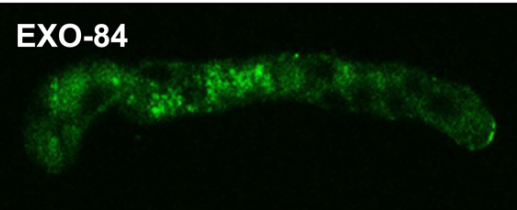
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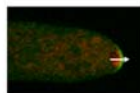
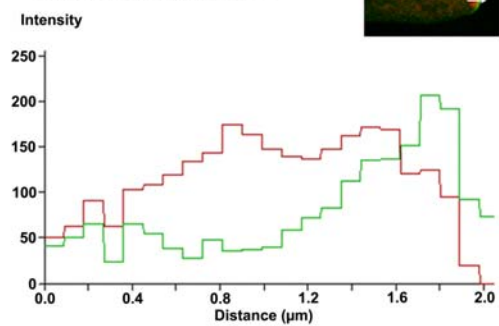
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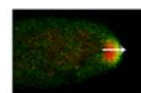
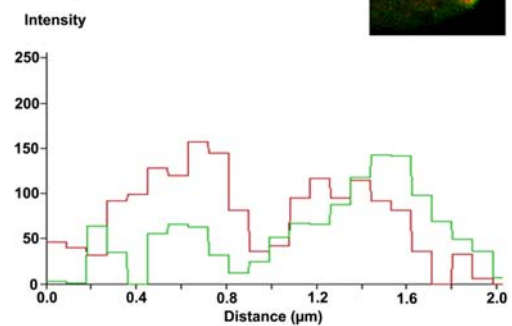
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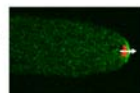
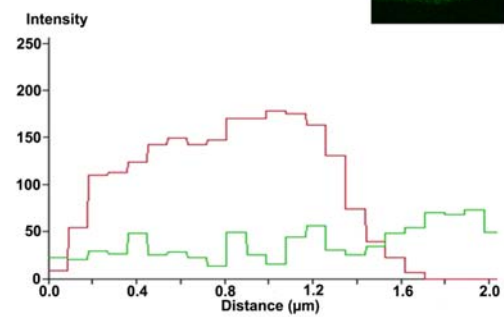
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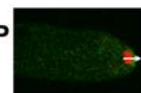
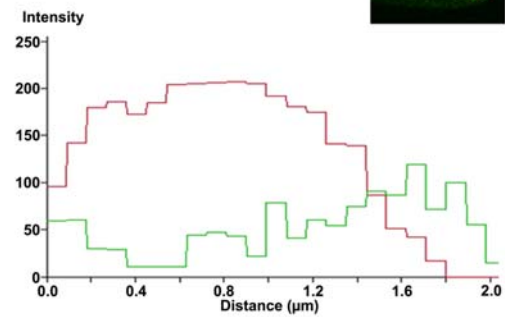
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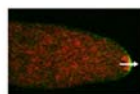
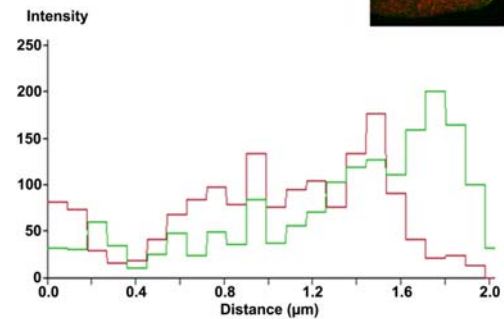
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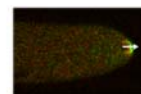
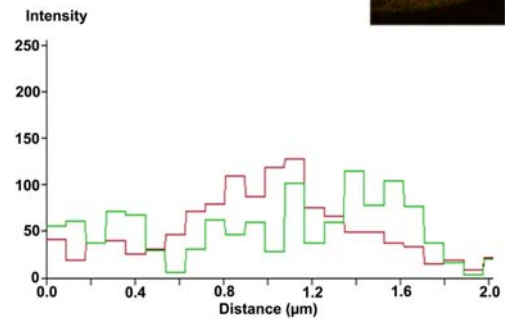
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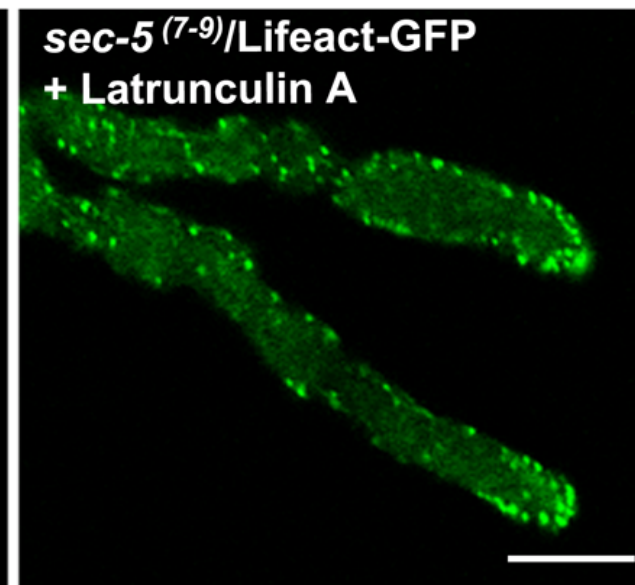
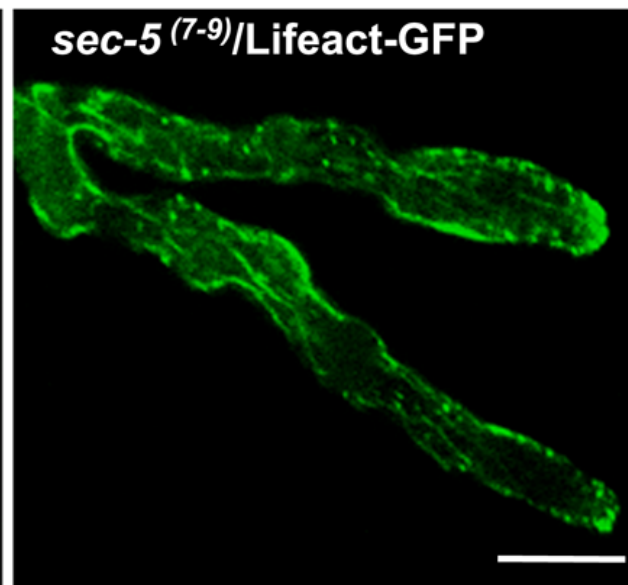
SEC-6-GFP/GS-1-mChFP



EXO-70-GFP/GS-1-mChFP



A



B

