Supplemental Figure 1. Fluorescent protein probes expressed in tobacco pollen.
(A) A pollen tube expressing YFP-RabA4b that labels transport vesicles. Inset shows a longitudinal tail of RabA4b labeling in a transformed pollen tube.
(B) A pollen tube expressing ARA6-YFP that labels endosomes.
(C) A pollen tube expressing YFP-ARA7 that labels endosomes.
(D) A pollen tube expressing YFP-mTalin that labels the actin cytoskeleton.
Left panels, YFP channel; right panels, transmitted light.
Scale bars = 20 μm.

Supplemental Figure 2. Pollen tube morphology and growth rate were not affected in pollen expressing fluorescent probes.
(A) Pollen tube width.
(B) Pollen tube growth rate.

Supplemental Figure 3. Effect of BFA treatment on transport vesicles labeled by YFP-RabA4b and FM4-64.
Pollen tubes expressing YFP-RabA4b treated with BFA at 15 min (A), 30 min (B) and 45 min (C). 5 min before imaging, 4 mM FM4-64 in DMSO was added to the medium. Top images were taken with the YFP channel, middle images were taken with the RFP channel. Merged images are shown at the bottom. Scale bar = 20 μm.

Supplemental Figure 4. Effect of Wortmannin treatment on transport vesicles labeled by YFP-RabA4b and FM4-64.
Pollen tubes expressing YFP-RabA4b treated with Wortmannin at 30 min (A), 60 min (B) and 90 min (C). 5 min before imaging, 4 mM FM4-64 in DMSO was added to the medium. Top images were taken with the YFP channel, middle images were taken with the RFP channel. Merged images are shown at the bottom. Scale bar = 20 μm.

Supplemental Figure 5. Effect of BFA treatment on pollen tubes expressing either ARA6-YFP (A) or YFP-ARA7 (B) and labeled with FM4-64. Top images were taken with the YFP channel and bottom images were taken with the RFP channel. Scale bar = 20 μm.

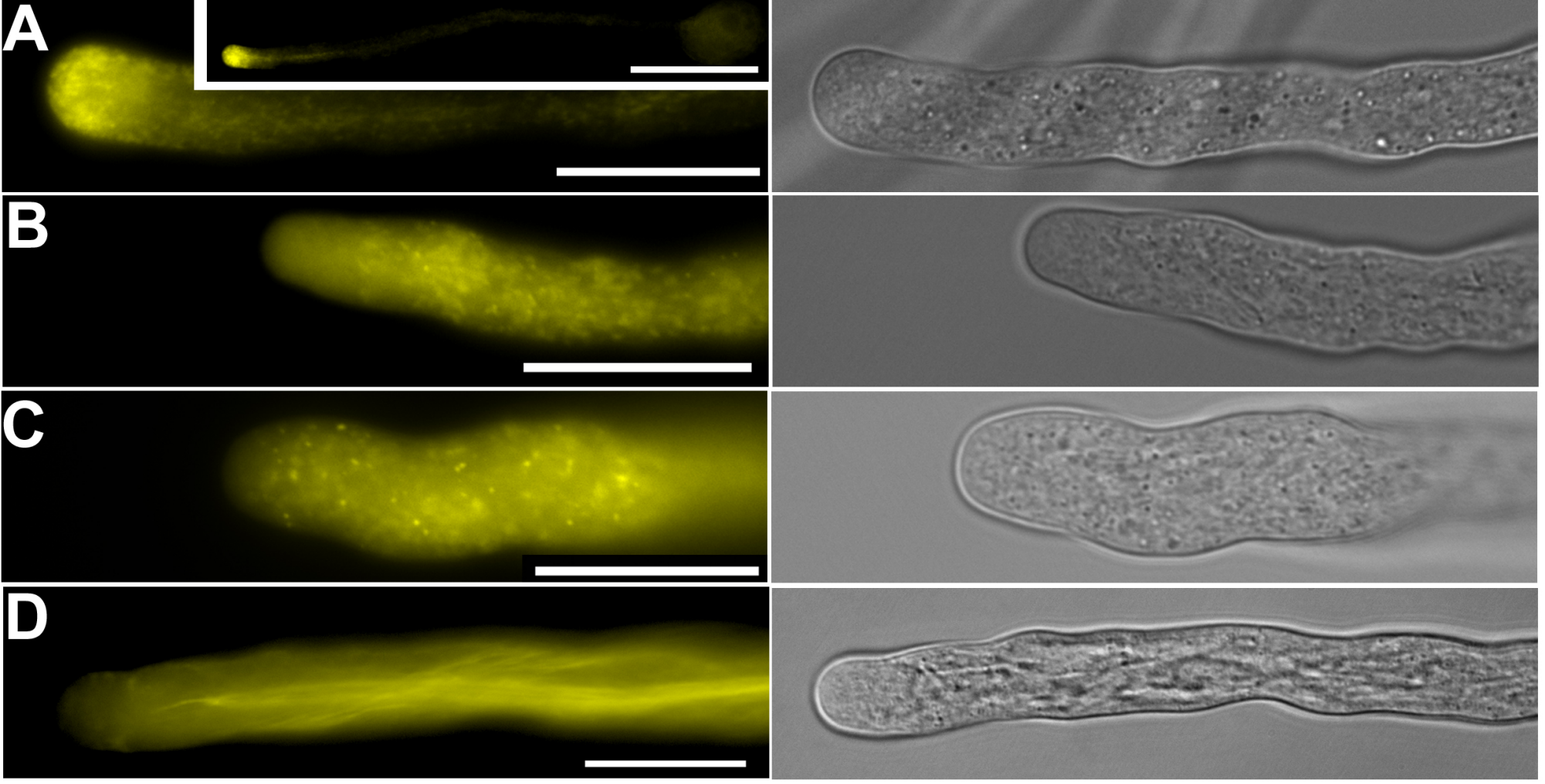
Supplemental Figure 6. Effect of Wortmannin treatment on pollen tubes expressing either ARA6-YFP (A) or YFP-ARA7 (B) and labeled with FM4-64. Top images were taken with the YFP channel and bottom images were taken with the RFP channel. Scale bar = 20 μm.

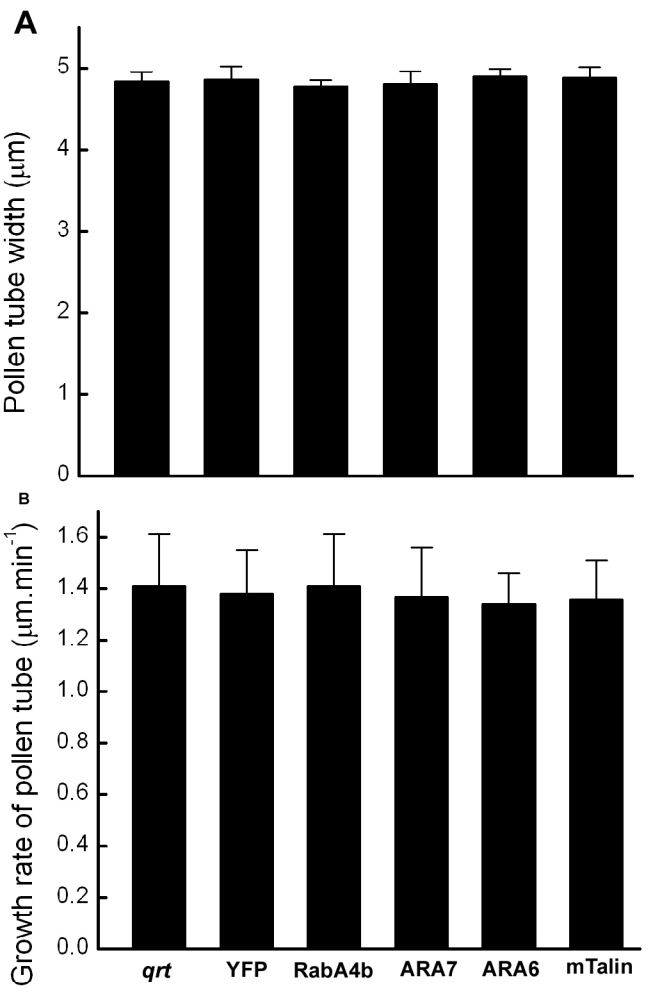
Supplemental Figure 7. Effects of BFA and Wortmannin treatments on pollen tubes co-expressing ARA6-CFP and YFP-ARA7. Top images were taken with the CFP channel; middle images with the YFP channel. Merged images are shown at the bottom. Scale bar = 20 μm.

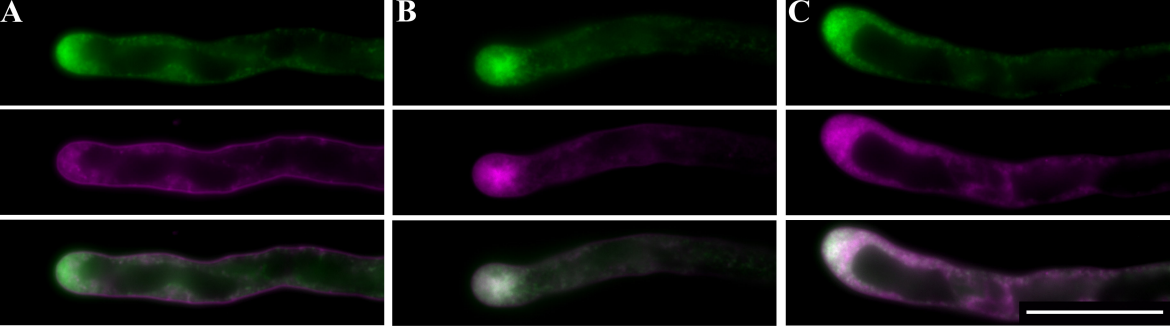
Supplemental Figure 8. The effects of actin disrupting drugs on the YFP-mTalin labeling pattern.
(A) A pollen tube treated with LatB for 30 min. Arrow indicates where the F-actin ring would be localized in non-treated tubes.
(B) A pollen tube treated with LatB for 60 min. Arrow indicates where the F-actin ring would be localized in non-treated tubes.
(C) A pollen tube treated with Jas for 10 min. Note that a few actin cables remained intact (shown with asterisk), while thick actin patches were already formed.
(D) A tube treated with Jas for 40 min. No actin cables were visible, and instead, thick actin patches were present throughout the tube.
Dotted lines show the contour of the apex of pollen tubes. Scale bar = 20 μm.

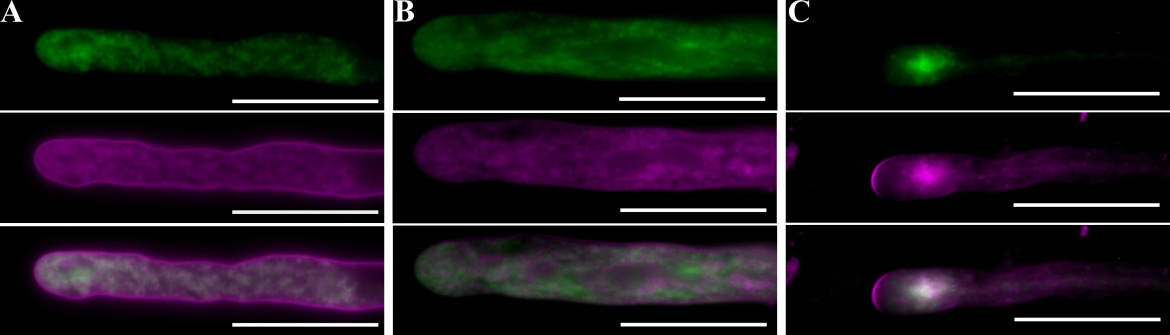
Supplemental Movie 1. Transport vesicles labeled by YFP-RabA4b in a growing control tube.
Supplemental Movie 2. Transport vesicles labeled by YFP-RabA4b in a pollen tube treated with BFA for 30 min.
Supplemental Movie 3. Transport vesicles labeled by YFP-RabA4b in a pollen tube treated with Wortmannin for 30 min.
Supplemental Movie 4. ARA7-positive endosomes treated with Wortmannin for 60 min.

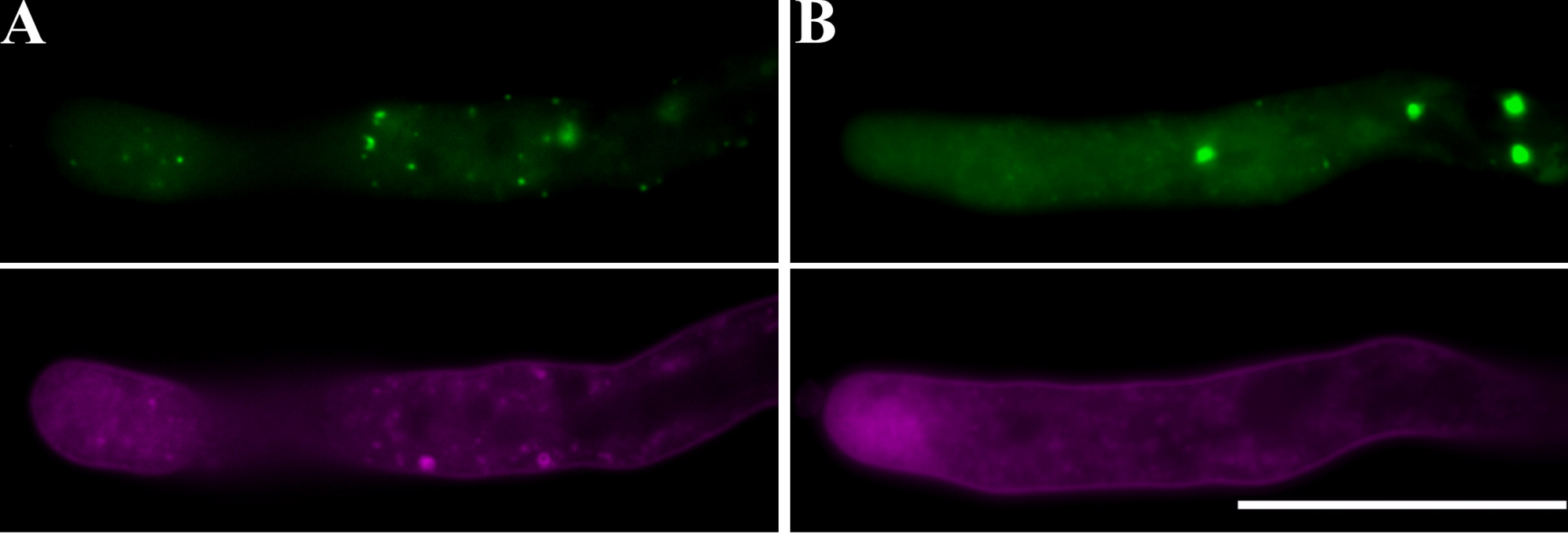
Supplemental Movie 5. ARA6-positive endosomes treated with BFA for 45 min.
Supplemental Movie 6. Transport vesicles labeled by YFP-RabA4b in a pollen tube treated with LatB for 20 min.
All movies are 30 frames taken at 30 sec intervals and play at 5 frames/sec.

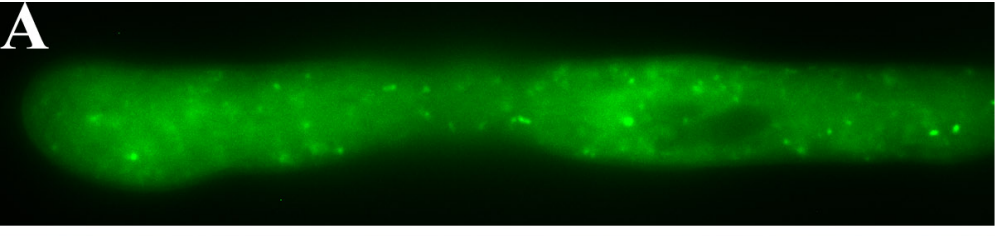
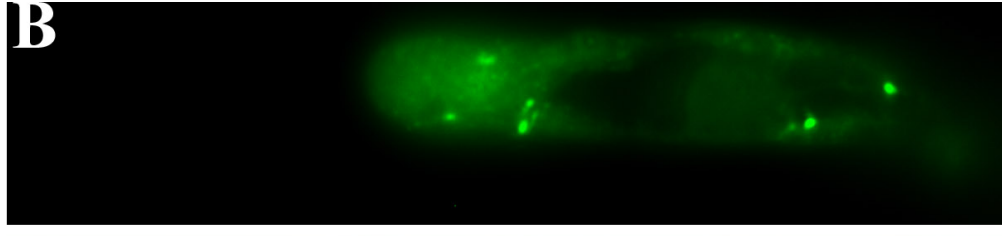

A
B
C
D


A
Pollen tube width (μm)
0
1
2
3
4
5
B
Growth rate of pollen tube (μm.min-1)
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
qrt
YFP
RabA4b
ARA7
ARA6
mTalin


A
B
C


A
B
C


A
B


A

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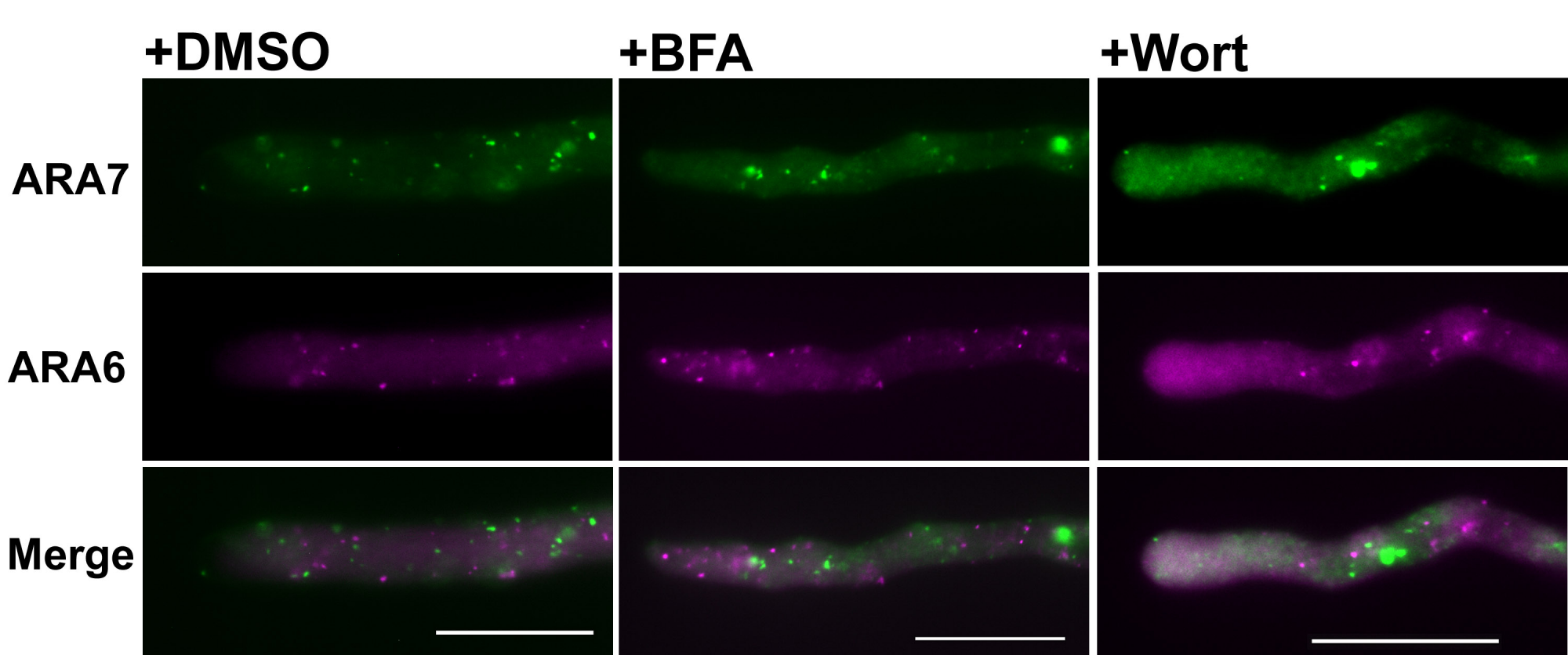
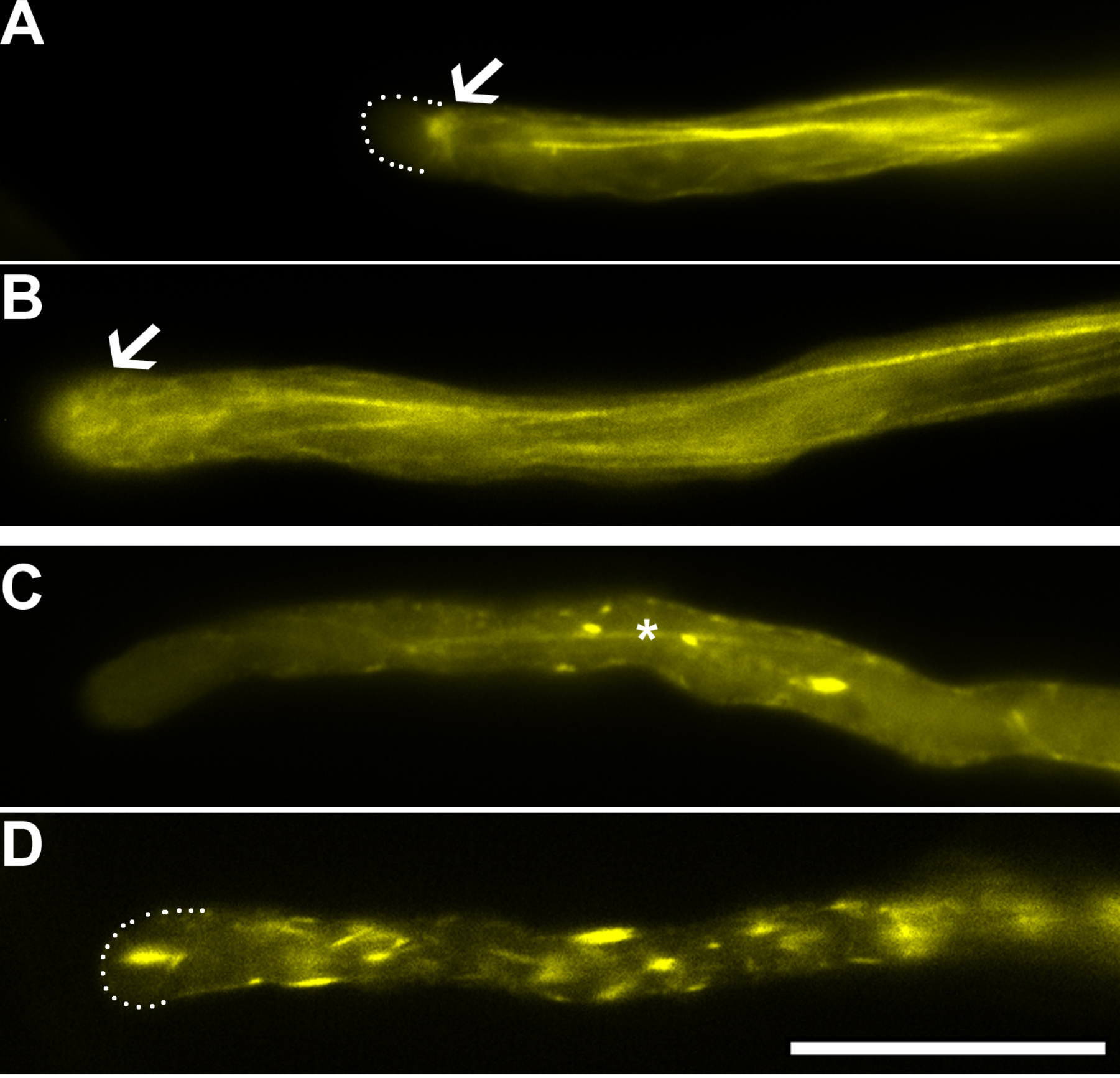

+DMSO
+BFA
+Wort
ARA7
ARA6
Merge


A
B
C
*
D