

SUPPLEMENTAL MATERIAL FOR BOWMAN ET AL.

TABLE 1. Primers and plasmids constructed for this study

<i>N.crassa</i> gene	5' primer	3' primer	Host vector	plasmid
<i>atg-8</i>	ccttacgcgtatgagatccaagtcaaggacgagc	ccttagatctcgcagtctcgaagtcgc	pMF334	ATG-8-dsRED
<i>cpy</i>	caactagtcacaatgaggatctcggctfc	catgttaaftaactgctcgaaccattcaccg	pMF272	CPY-GFP
<i>pho-8</i>	cccttctagacgcaatgtaagtgaag	gtttcccgggcacatggccataaagt	pMF272	PHO-8-GFP
<i>rab-2</i>	ccttacgcgtatgctcctcattacctgg	caa aagatctcaacaacacctccataacc	pMF334	RAB-2-dsRED
<i>rab-4</i>	ccttacgcgtatgacaagacctagggcgtc	cagaagatctcaacaacaacctgttctctcc	pMF334	RAB-4-dsRED
<i>rab-5A</i>	ccttacgcgtatggccactcgtggacctc	caaatctagatcagcaagcacaaggccctc	pMF334	RAB-5A-dsRED
<i>rab-5B</i>	ccttacgcgtatggccgatacgaacgcg	cacaatctagactttaacagcgcacaccttc	pTSL43B	RAB-5B-mCherry
<i>rab-6</i>	ccttacgcgtatcacaatggcgcaagcagggcgtc	caaaagatctttaacaagcgcaccttctg	pTSL43B	RAB-6-mCherry
<i>rab-7</i>	ccttacgcgtatgtctcaggaagaaggffc	caaaagatcttagcacgcgcattccatccc	pMF334 pTSL43B	RAB-7-dsRED RAB-7-mCherry
<i>rab-8</i>	ccttacgcgtatgtcagtaacaggaattacg	cagaagatctttaacagcacttgcggccgga	pTSL43B	RAB-8-mCherry
<i>rab-11</i>	ccttacgcgtatggccaacgacgagtacgat	caaaagatcttaacagcagccagactcgc	pMF334	RAB-11-dsRED
<i>tlg-1</i>	ctttacgcgtatgtcctccacgaacgaggag	ccctagatctcaaatgagcaataaaaccaacaac	pMF334	TLG-1-dsRED
<i>vma-1</i>	gcttaattaacatgctccgtaagccttg	ctttgcggcgcctactcatcaatgacagagcg	pCCG::N-GFP	VMA-1-GFP
<i>vtc-4</i>	ccaacgcgtatgaagttcggcgaacagctc	ccaatctagagccaaacctctgccttc	pMF334	VTC-4-dsRED

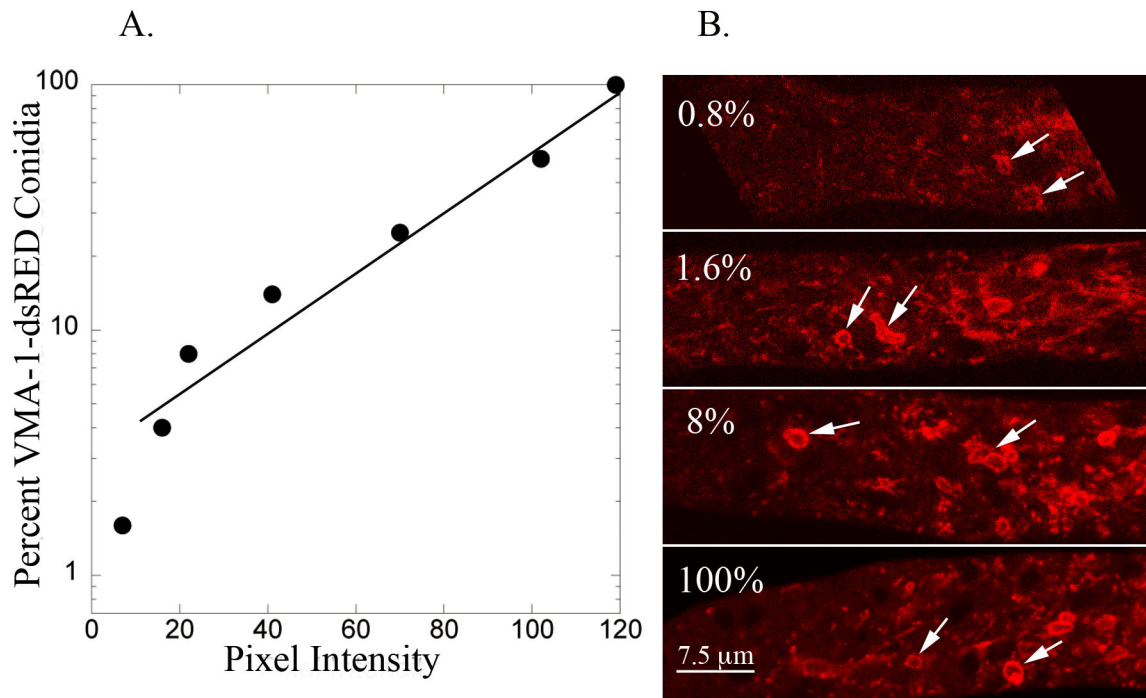


FIG S1. Expression of VMA-1-dsRED in heterokaryons formed with the wild type and the VMA-1-dsRED. The ratio of nuclei was varied by co-inoculating with different ratios of conidia obtained from the wild type strain 74A and the VMA-1-dsRED strain. The intensity of red fluorescence was quantified as described in Materials and Methods. (A) The pixel intensity in the region with the tubular vacuolar network was measured for 6 different heterokaryons (0.8% - 50% VMA-1-dsRED conidia) and for the VMA-1-dsRED homokaryon. (B) Images of the VMA-1-dsRED homokaryon and three of the heterokaryons are shown with arrows pointing to examples of the PVCs. The images of the heterokaryons were digitally enhanced to make the red fluorescence brighter. Note that the measurement of pixel intensity (Panel A) was done before the digital enhancement.

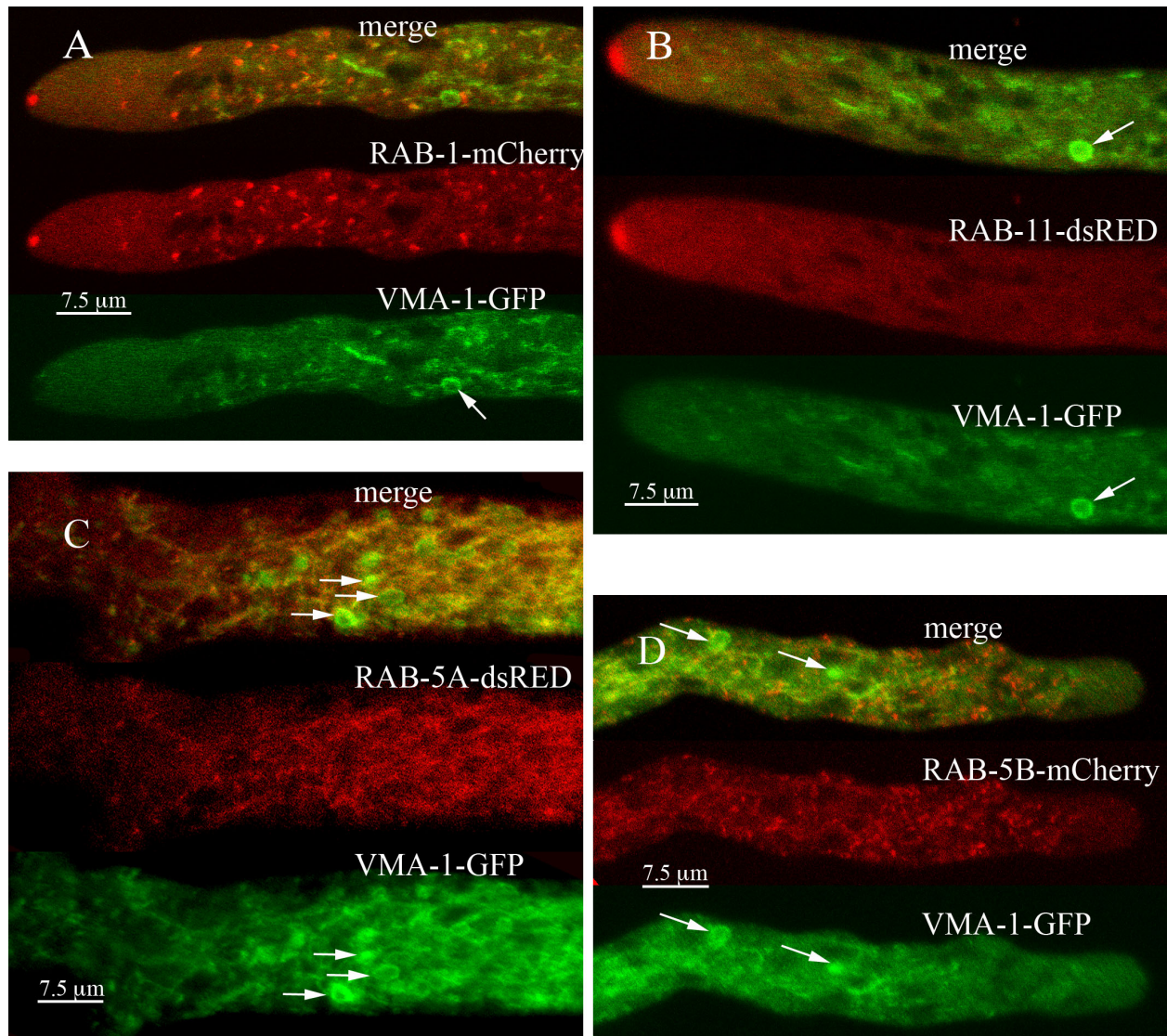


FIG S2. Localization of the Rab-1 GTPase (A), the rab-11 GTPase (B), the rab-5A GTPase (C), and the rab-5B GTPase (D). Each was visualized in a heterokaryon with VMA-1-GFP. Note that RAB-1-mCherry and RAB-11-dsRED are abundant at the hyphal tip. Arrows point to examples of the PVCs.

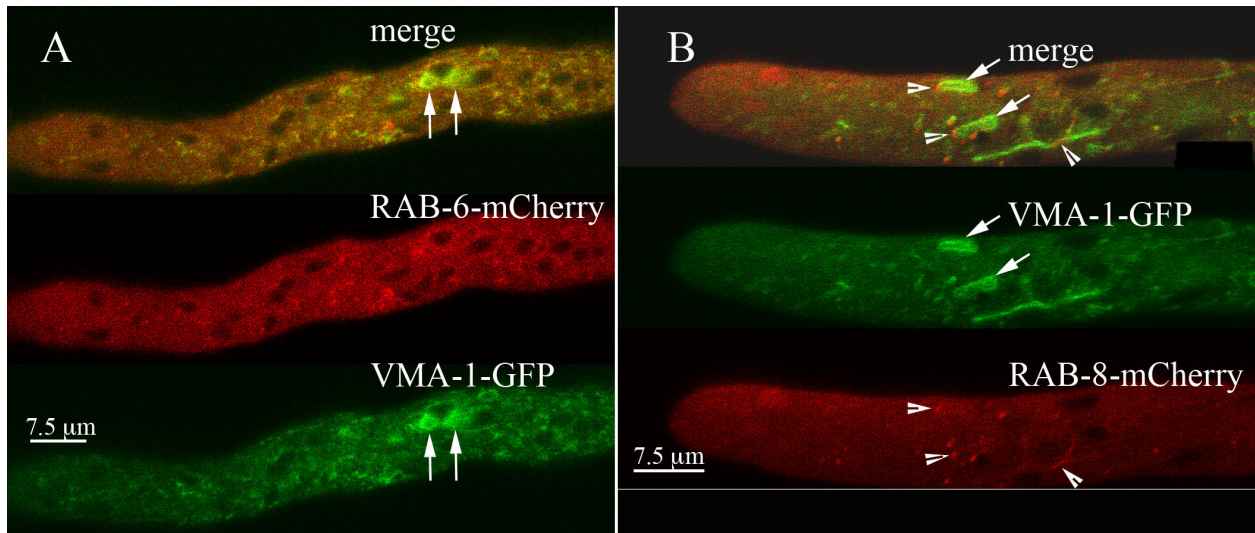


FIG S3. Localization of the Rab-6 GTPase (A) and the rab-8 GTPase (B). Each was visualized in a heterokaryon with VMA-1-GFP. Arrows point to examples of the PVCs. Rab-8-mCherry is also found in the Spitzenkorper (48) which is not visible in this photo.

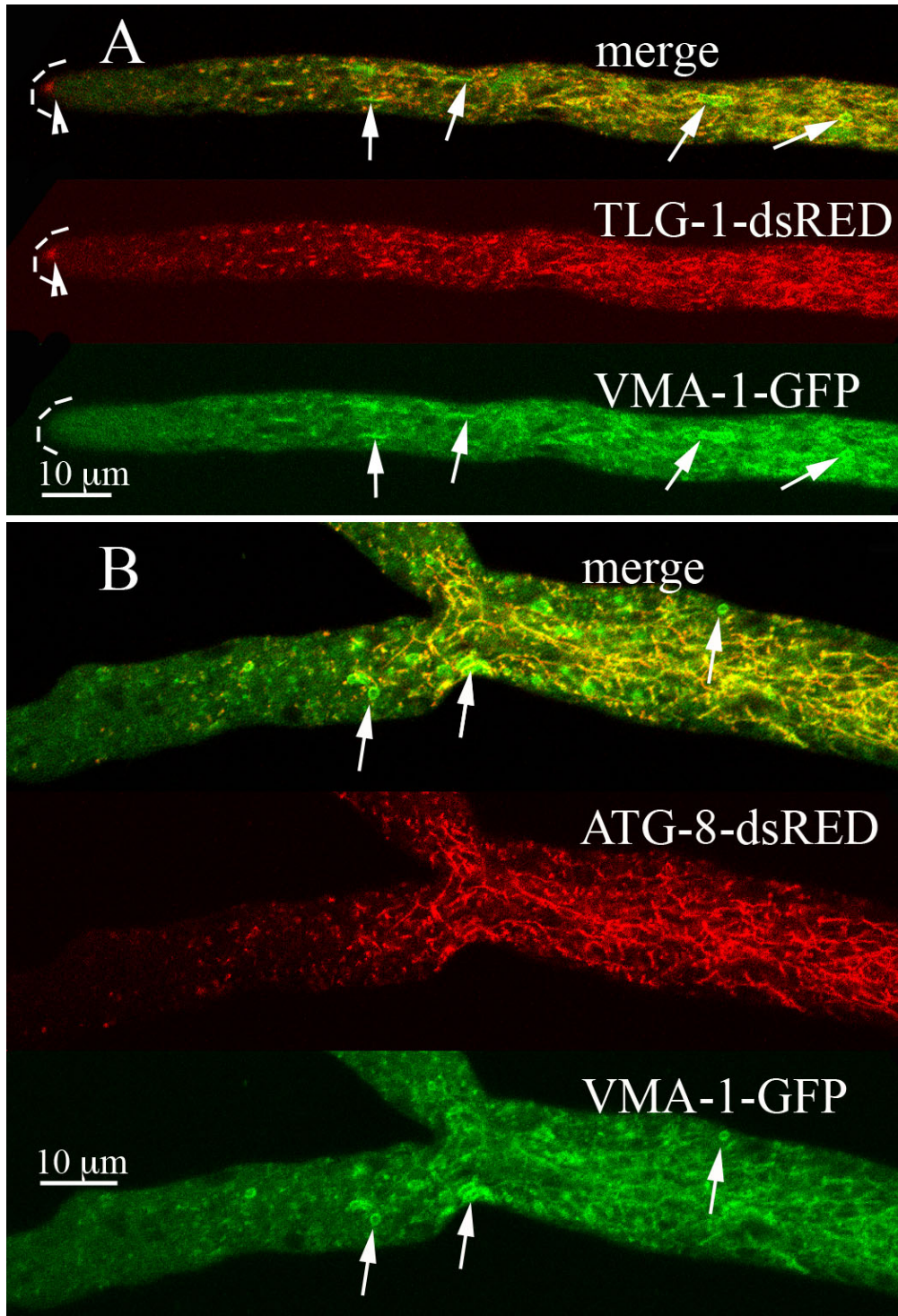


FIG S4. Localization of TLG-1 (A) and ATG-8 (B). Each was visualized in a heterokaryon with VMA-1-GFP. Arrows point to examples of the PVCs.

LEGENDS TO SUPPLEMENTAL MOVIES

Movie #1. PVCs are variable in size and can change shape quickly. The hypha was expressing VMA-1-dsRED. Some of the PVCs, marked by arrows appeared to form protrusions and exhibit blebbing. The tubular vacuolar network comes into view in the second half of the movie. Successive frames were taken at 5.4 sec intervals in all movies.

Movie #2. PVCs were visible in the *ad-3B* strain growing on a low concentration of adenine (0.1 mM). The first arrow points to a typical spherical PVC. The tubular vacuolar network was also strongly fluorescent. The second arrow points to a PVC that formed a long projection.

Movie #3. PVCs can form tubular projections which appear to separate. The hypha was expressing RAB-7-dsRED. Arrows point to examples of PVCs.

Movie #4. Some PVCs were very large. The hypha is expressing RAB-7-dsRED. The image of the large PVC as it moved up and down in the focal plane suggests it had a spherical shape. Note the split membrane visible at the end of the movie.