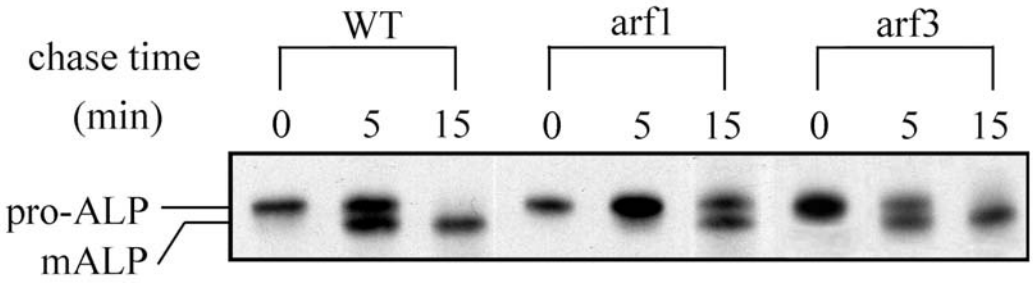
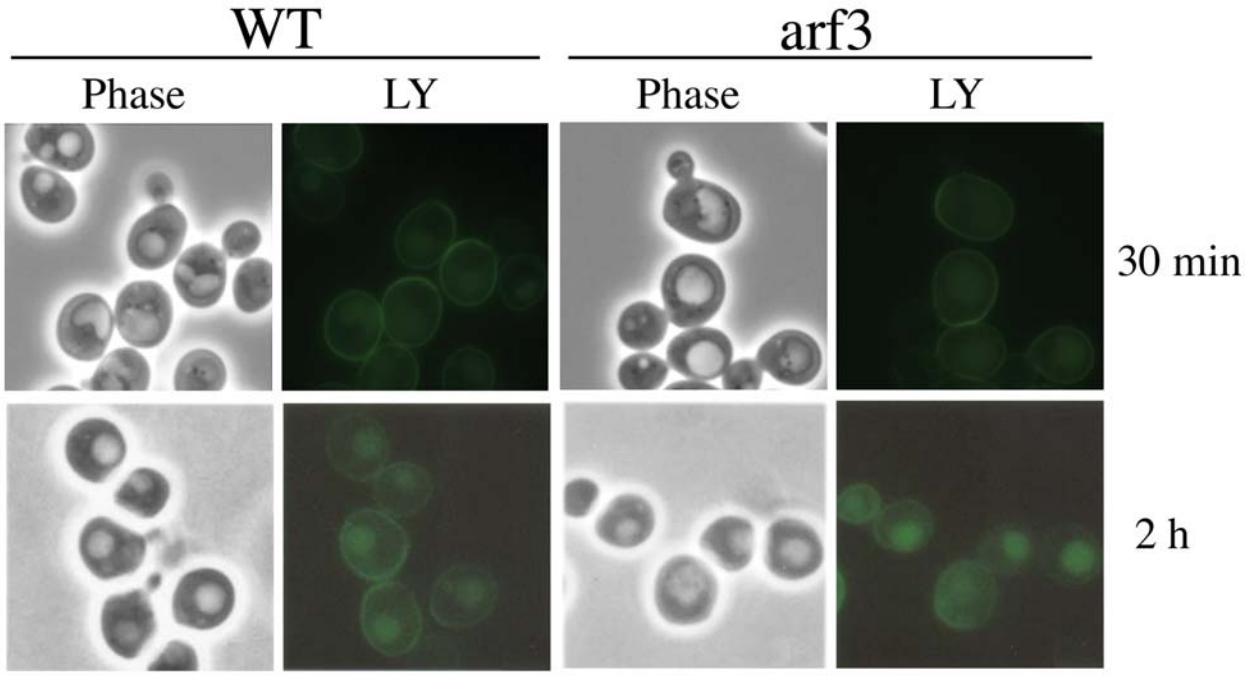
Supplementary Data

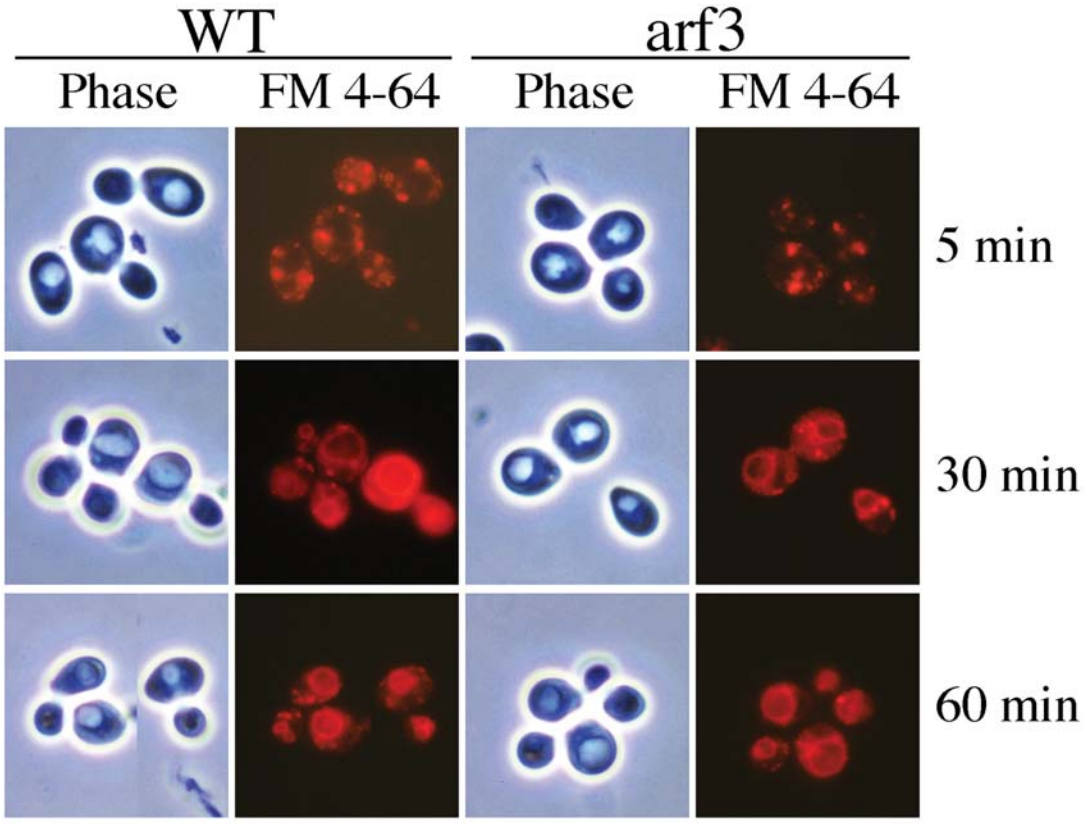
Figure 1. Effect of ARF3 on sorting of vacuolar ALP. Wild-type (ARF1/ARF3), *arf3* mutant, and *arf1* mutant cells were grown and radiolabeled with ^{35}S-labeled methionine and cysteine at 37^{o}C. Immunoprecipitates were prepared as described in Materials and Methods at the indicated time points. pro-ALP is the proenzyme form, and mALP is the mature form of the enzyme in the vacuole.

Figure 2. Accumulation of Lucifer Yellow in vacuole in wild-type and *arf3* mutant cells. Lucifer Yellow was used to assess fluid-phase endocytosis in wild-type and *arf3* mutant cells. Wild type or *arl3* mutant was grown at 30^{o}C and transferred to fresh YPD before addition of LY for 30 min or 2 h and then viewed by phase-contrast (right) and fluorescence (left) microscopy.

Figure 3. Endocytosis of FM 4-64 by wild-type and *arf3* mutant cells. Cells were grown at 30^{o}C, transferred to fresh YPD at 4^{o}C before addition of FM 4-64, and viewed by phase-contrast and fluorescence microscopy at the indicated times after return to incubation at 30^{o}C.


WT
arf1
arf3
chase time
(min)
0
5
15
0
5
15
0
5
15
pro-ALP
mALP


WT
arf3
Phase
LY
Phase
LY
30 min
2 h


WT
arf3
Phase
FM 4-64
Phase
FM 4-64
5 min
30 min
60 min