SUPPLEMENTAL FIGURE LEGENDS

Supplementary figure 1: The non-Golgi structure labeled by ARF1 partially overlap with FM4-64. To determine whether ARF1 is distributed to endocytic structures in tobacco as it has been demonstrated in Arabidopsis and onion cells, we labeled tobacco leaf epidermal cells co-expressing ARF1-GFP and the Golgi marker, ST-YFP (Brandizzi et al., 2002a) with FM4-64 (Bolte et al., 2004). This triple labeling approach provides the advantage of distinguishing the population of ARF1 bound to non-Golgi structures from the ARF1 bound to the Golgi apparatus. The inclusion of the Golgi marker in this experiment is an important control as FM4-64 has been reported to label the Golgi apparatus in some plant cell species, including BY-2 cells and *Nicotiana benthamiana* (Bolte et al., 2004; Haupt et al., 2005). We found that a population of ARF1-GFP was localized to the Golgi, as expected. In addition, we found that the ARF1 non-Golgi structures co-localized with FM4-64 positive organelles (arrowheads), which were distinct from structures labeled only with FM4-64 (arrows). These results indicate that ARF1 is partially distributed to endocytic structures in agreement with previous findings in different plant cell types (Xu and Scheres, 2005). Scale bar = 5 μm.

Supplementary figure 2: YFP-GDAP1 and ARF1-GFP maintain fluorescence without BFA treatment. Time-lapse confocal micrographs demonstrate that there is no loss of fluorescence in a live tobacco epidermal cell coexpressing YFP-GDAP1 and ARF1-GFP over time. Representative control images are shown at comparable time points to the BFA treatment data presented in Figure 3. The time course of fluorescence was quantified in Figure 3C. The time (s) is indicated in the top right-hand corner. Scale bar = 5 μm.

Supplementary figure 3: εCOP-YFP and ARF1-GFP maintain fluorescence without BFA treatment. Time-lapse confocal micrographs demonstrate that there is no loss of fluorescence in a live tobacco epidermal cell coexpressing εCOP-YFP and ARF1-GFP over time. Representative control images are shown at comparable time points to the

BFA treatment data presented in Figure 6. The time course of fluorescence was quantified in Figure 6C. The time (s) is indicated in the top right-hand corner. Scale bar = 5 μm.

Supplementary figure 4: εCOP-YFP and CFP-GDAP1 maintain fluorescence without BFA treatment. Time-lapse confocal micrographs demonstrate that there is no loss of fluorescence in a live tobacco epidermal cell coexpressing εCOP-YFP and CFP-GDAP1 over time. Representative control images are shown at comparable time points to the BFA treatment data presented in Figure 7. The time course of fluorescence was quantified in Figure 7C. The time (s) is indicated in the top right-hand corner. Scale bar = 5 μm.