Supplementary Material

Golgi enlargement in Arf-depleted yeast cells is due to altered dynamics of cisternal maturation

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Fig. S1. Reversion or suppression of the *nmt1* mutation restores the wild-type appearance of late Golgi cisternae.

The methods and terminology are as in Fig. 1. Scale bar, $1 \mu m$.



Fig. S2. The *arf1* Δ mutation does not promote tER site coalescence.

Wild-type (WT) or $arf1\Delta$ cells expressing the tER site marker Sec13-GFP were imaged by fluorescence microscopy. *Left:* representative cells are shown. Scale bar, 2 µm. *Right:* the average number of tER sites per cell was quantified for 25-30 cells from each strain. Error bars indicate s.e.m.



Fig. S3. The *arf1* Δ mutation has only a modest effect on early Golgi cisternae. (A) Fluorescence images show representative wild-type (WT) and *arf1* Δ cells expressing GFP-Vrg4. Scale bar, 1 µm. (B) The average volume of early Golgi cisternae was measured as in Fig. 1 for WT and *arf1* Δ strains.

Α



В





Mutant: ~4 early cisternae, ~2 late cisternae



Fig. S4. Graphical representation of the Golgi cisternal life cycle in wild-type and $arf1\Delta$ cells.

(A) Time arrow depiction of a yeast Golgi cisterna, with brackets marking the early Golgi persistence time p_E and the late Golgi persistence time p_L .

(**B**) Simplified model of Golgi cisternal cycles in a wild-type cell and an $arf1\Delta$ cell. Time proceeds along the horizontal axis, and each arrow represents a different cisterna. Compared to the wild-type cell, the $arf1\Delta$ cell has twice as large a value of p_E and half as large a value of the maturation frequency m. At any given time point, the $arf1\Delta$ cell has about the same number of early Golgi cisternae as the wild-type cell but only about half as many late Golgi cisternae. Although this model does not capture the full complexity of Golgi maturation in wild-type and $arf1\Delta$ cells, it conveys the key insights described in this study.



Movie 1.



Movie 2.



Movie 3.



Movie 4.

Movie 1. Tomographic models of late Golgi cisternae in wild-type cells.

Models generated as described in Fig. 2D were assembled using IMOD software (<u>http://bio3d.colorado.edu/imod/</u>) and animated using ImageJ. The models show portions of three late Golgi cisternae from a single thick section.

Movies 2 and 3. Tomographic models of late Golgi cisternae in *arf1* Δ cells.

The procedure was the same as in Movie 1. Movie 2 corresponds to the bottom panels of Fig. 2D, and Movie 3 shows two additional cisternae from a different cell.

Movie 4. Golgi maturation and homotypic fusion in an *arf* 1Δ cell.

Early Golgi cisternae were tagged with GFP-Vrg4, and late Golgi cisternae were tagged with Sec7-DsRed. A representative cell was imaged by 4D confocal microscopy with a Zeiss LSM 780 for 3.5 min, with Z-stacks collected at 3.65-sec intervals. Times are indicated in min:sec format, rounded down to the nearest second. In Fig. 4A, frames from this movie illustrate the homotypic fusion of two early Golgi cisternae.