Supplemental material

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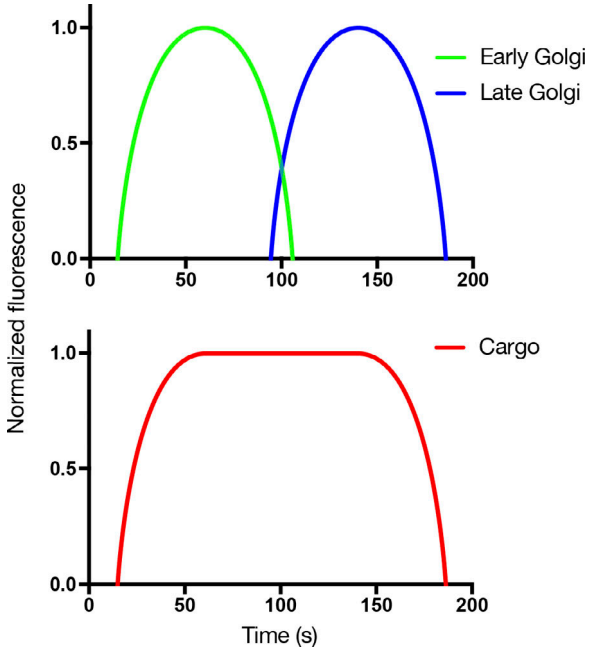


Figure S1. **Predicted cargo fluorescence signal in a maturing yeast Golgi cisterna.** Green and blue represent early and late resident Golgi proteins, respectively, and red represents the fluorescent secretory cargo. In the simplest envisioned scheme, the fluorescent cargo remains within a cisterna as it matures, so the cargo signal is constant before, during, and after the early-to-late transition.

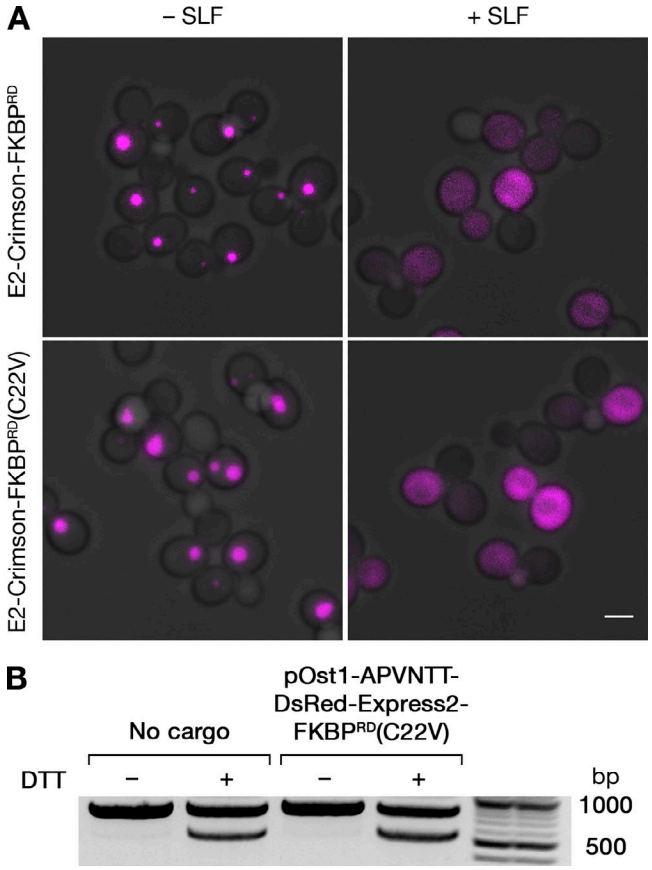


Figure S2. **Aggregated tetramers in the yeast cytosol and ER. (A)** Reversible aggregation of a cytosolic construct containing a dimerizing FKBP variant with the C22V mutation. Cells expressing cytosolic aggregates of either E2-Crimson-FKBPRD (Barrero et al., 2016) or E2-Crimson-FKBPRD(C22V) were grown to mid-log phase and imaged by confocal microscopy before and 3 min after addition of SLF. Shown are representative projected Z-stacks. Scale bar, 2 µm. **(B)** Lack of UPR activation by aggregated cargo in the ER. A parental *pdr1Δ pdr3Δ vps10-104* strain and a derivative strain that expressed pOst1-APVNTT-DsRed-Express2-FKBPRD(C22V) to generate ER-localized aggregates were grown overnight with shaking in minimal medium at 23°C to an OD_{600} of 0.6–0.8. Each culture was then split, and one half was mock treated while the other half was treated with 8 mM DTT for 30 min to activate the UPR. RNA was isolated from the cells, and RT-PCR was performed to measure the splicing of *HAC1* mRNA. PCR products were separated by electrophoresis in a 1% agarose gel together with the 2-Log DNA Ladder (New England Biolabs; N0469S). The darker bands in the DNA ladder are at 500 and 1,000 bp. Unspliced *HAC1* mRNA is 819 bp, and the spliced form is 567 bp.

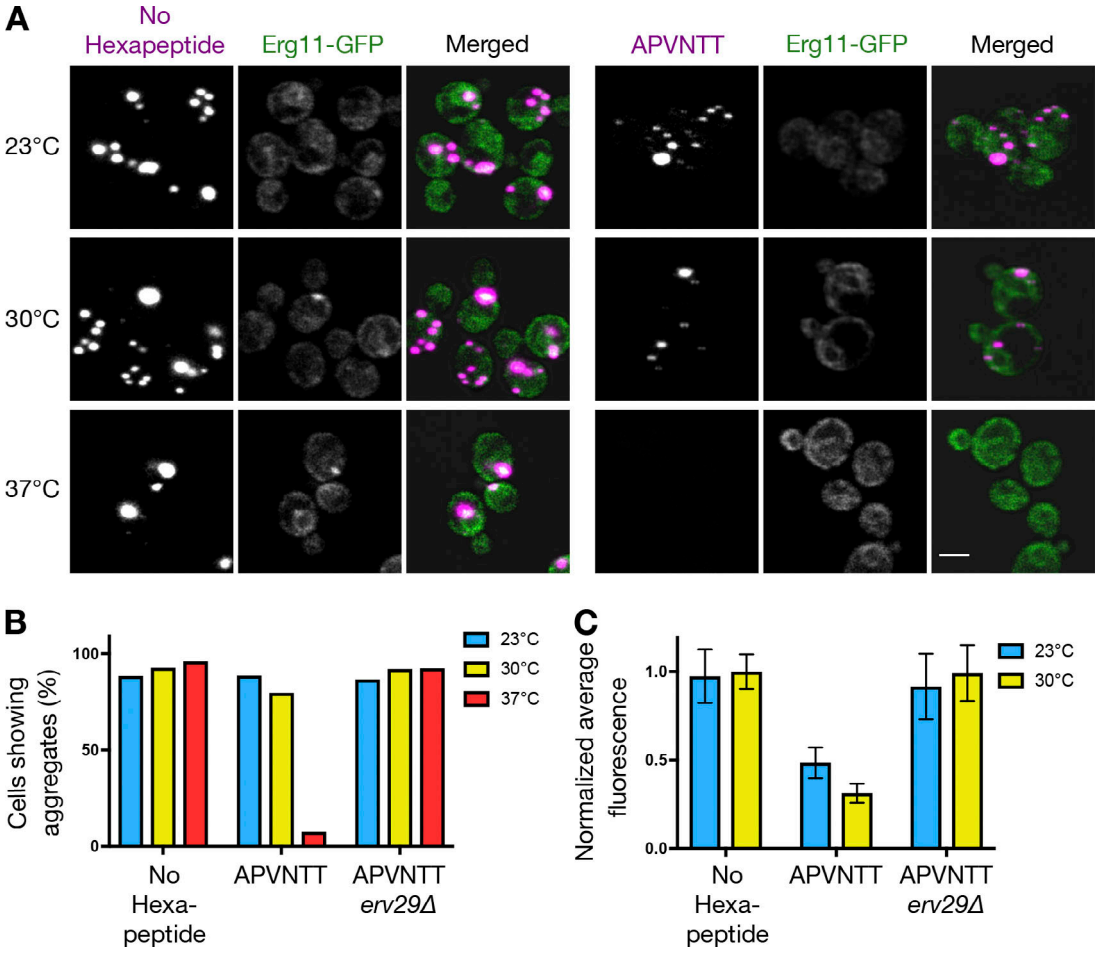


Figure S3. **Effect of temperature on the ER aggregation of cargo variants. (A)** Projected confocal Z-stacks of yeast cells expressing fluorescent cargo constructs after growth at the indicated temperatures. "APVNTT" indicates that an additional hexapeptide was present at the N terminus of the mature DsRed-Express2-FKBPRD(C22V) construct after cleavage of the signal sequence. The ER was labeled with Erg11-GFP. **(B)** Quantification of the percentages of cells with visible ER aggregates at different temperatures. For the samples in A, confocal projections of cells were manually scored for the presence or absence of visible aggregates. Measurements were taken from at least 40 cells per condition. **(C)** Average fluorescence of aggregates. For the samples in A, aggregates visible in confocal projections were selected using the Analyze Particles tool in ImageJ, and the fluorescence in a given cell was measured. Measurements were taken from at least 50 cells per condition. Error bars represent SEM.

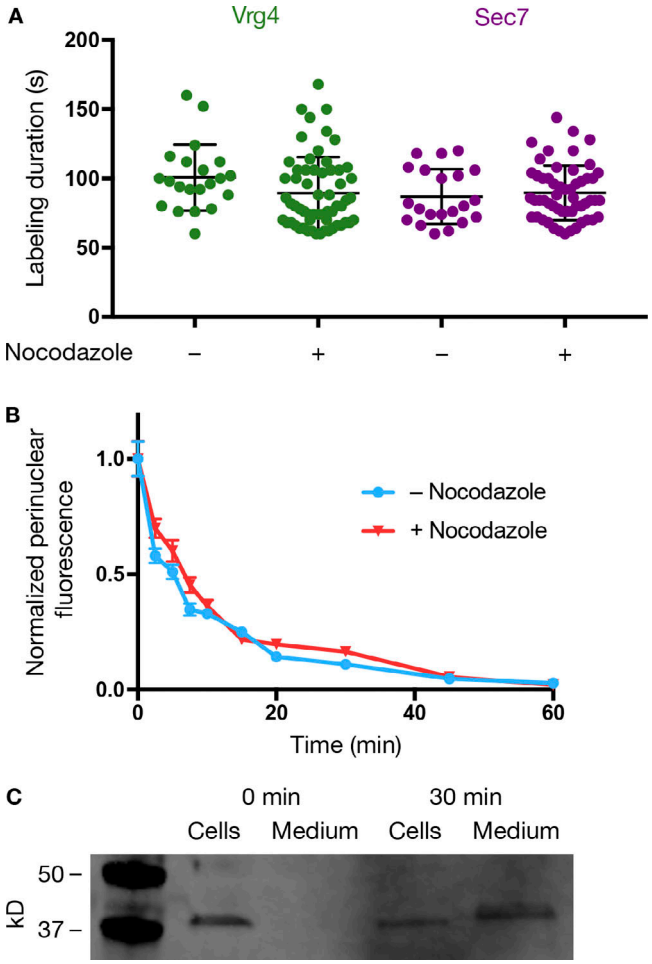


Figure S4. **Insensitivity of cisternal maturation rates and fluorescent secretory cargo traffic to nocodazole treatment. (A)** Scatter plot showing the durations of early and late Golgi maturation stages in untreated and nocodazole-treated cells. Cells expressing either GFP-Vrg4 (early Golgi) or Sec7-GFP (late Golgi) were grown to mid-log phase, either mock treated or treated with nocodazole for 2 h, and imaged by 4D confocal microscopy. For a given cisterna, the duration of an early or late Golgi stage was determined by measuring the time between the first and last video frames in which the cisterna visibly displayed the relevant Golgi marker. Each scatter plot shows the mean duration of a Golgi stage and the standard deviation. **(B)** Comparison of ER export rates for the fluorescent secretory cargo in the presence or absence of nocodazole. The experiment was performed as in Fig. 2 (B and C), except that cells expressing the APVNTT-DsRed-Express2-FKBPRD(C22V) cargo were either mock treated or treated with nocodazole for 2 h before SLF addition and imaging. **(C)** Immunoblot showing the appearance of the cargo in rich medium in the presence of nocodazole. The experiment was performed as in Fig. 3 C, except that the cells were treated with nocodazole for 2 h before SLF addition. Numbers represent molecular weights of reference markers.

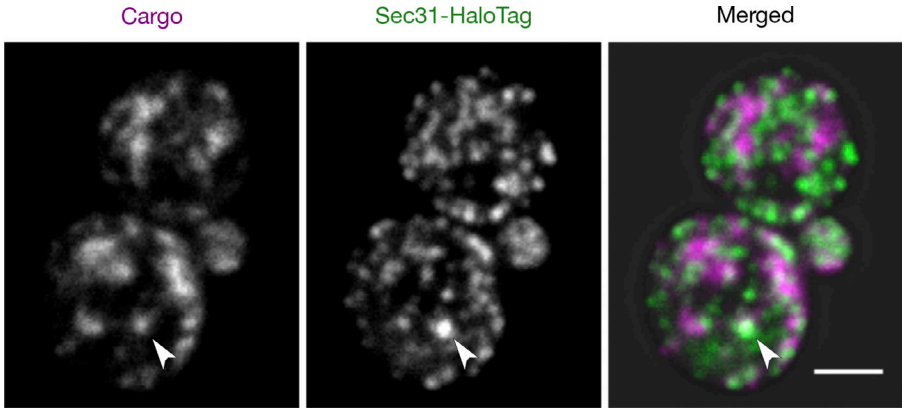
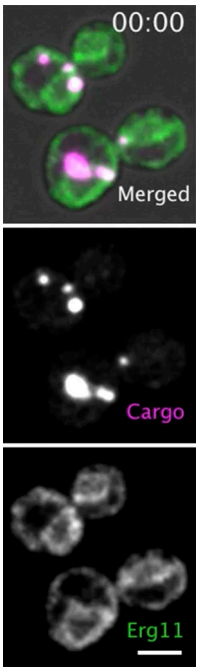
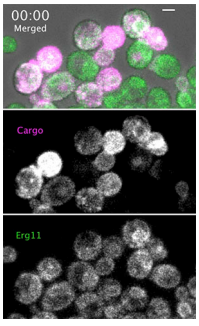


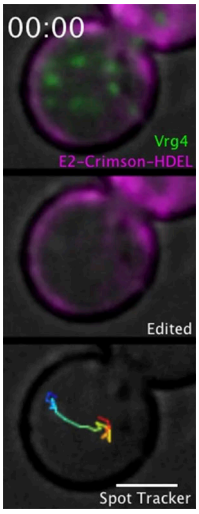
Figure S5. **Association of newly exported cargo with a subset of the ERES.** A strain expressing the APVNTT-DsRed-Express2-FKBPRD(C22V) cargo and the ERES marker Sec31-HaloTag was grown to mid-log phase, labeled with JF_{646}, treated with SLF for 2 min to trigger cargo solubilization and export, and fixed. The cells were compressed on a coverslip and imaged on a confocal microscope. This image is a representative projected Z-stack. The arrowhead marks an example of concentrated cargo in the vicinity of a single large ERES. Scale bar, 2 µm.



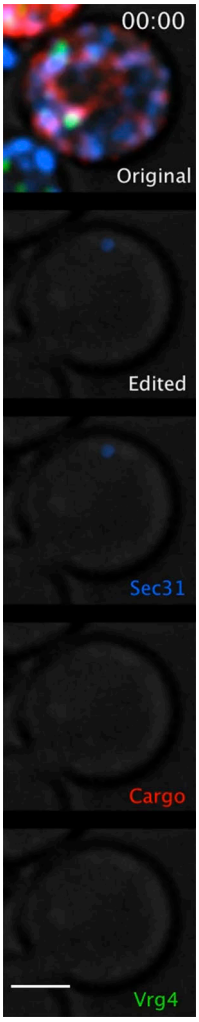
Video 1. **Dissolution of cargo aggregates in the ER lumen.** Cells expressed the DsRed-Express2-FKBPRD(C22V) cargo, together with Erg11-GFP to mark the ER. Medium that contained SLF was added while imaging on a confocal microscope, with a Z-stack collected every 2 s. The top panel merges the cargo, Erg11-GFP, and bright-field channels, while the middle panel is the cargo alone and the bottom panel is the Erg11-GFP channel alone. The video shows projections of 10 optical sections from the centers of the cells. Scale bar, 2 µm. Frames from this video are shown in Fig. 2 A.



Video 2. **Quenching of periplasmic cargo fluorescence by low pH.** Cells expressed the APVNTT-DsRed-Express2-FKBPRD(C22V) cargo, together with Erg11-GFP to mark the ER. A culture was grown to mid-log phase in NSD buffered at pH 6, treated with SLF for 30 min to allow secretion of the cargo, and mounted in a flow chamber. Unbuffered NSD (pH ∼4) was added while imaging on a confocal microscope, with a Z-stack collected every 3 s. The top panel merges the cargo, Erg11-GFP, and bright-field channels, while the middle panel is the cargo alone and the bottom panel is the Erg11-GFP channel alone. The video shows projections of the full Z-stacks. Scale bar, 2 µm. Frames from this video are shown in Fig. 3 E.



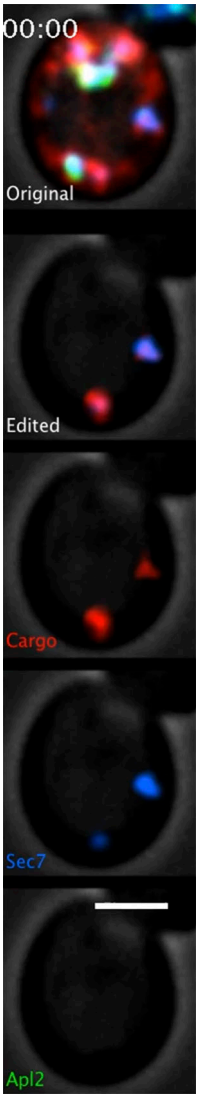
Video 3. **Localization of a typical early Golgi cisterna relative to the cortical ER in a nocodazole-treated cell.** Cells expressed GFP-Vrg4 to mark early Golgi cisternae, together with E2-Crimson-HDEL to mark the ER. After treatment for 2 h with nocodazole, the daughter was imaged on a confocal microscope, with a Z-stack collected every 2 s. The top panel shows projections of eight optical sections from the center of the cell. The middle panel was edited to show only the cisterna being tracked. The bottom panel shows the results of analyzing the movements of that cisterna with the TrackMate plugin, with the start position marked in blue and the end position marked in red. Bright-field images of the cell are present in all panels. Because only the center of the cell is shown, the cortical ER appears primarily as a ring around the periphery of the cell, but some of the projections also include cortical ER signal from the top or bottom of the cell. Scale bar, 2 µm. Frames from this video are shown in Fig. 4 (A and B).



Video 4. **Concentration of exported secretory cargo in a newly forming Golgi cisterna near an ERES.** Cells expressed the APVNTT-DsRed-Express2-FKBPRD(C22V) cargo, together with Sec31-HaloTag to mark ERES and GFP-Vrg4 to mark the early Golgi. A culture was grown to mid-log phase, labeled with JF_{646}, washed, and mounted in a flow chamber. Medium that contained SLF was added while imaging on a confocal microscope, with a Z-stack collected every 2 s. Cells were exposed to SLF ∼45 s before the beginning of the video. The top panel shows the complete video, with projections of the full Z-stacks. The second panel shows an edited video of a representative cargo-containing structure and the associated ERES and early Golgi structures. The bottom three panels show the individual fluorescence channels. Bright-field images of the cell are present in all panels. Scale bar, 2 µm. Frames from this video are shown in Fig. 5 A.

Video 5. **Continuous presence of the fluorescent secretory cargo within maturing Golgi cisternae.** Cells expressed the APVNTT-DsRed-Express2-FKBPRD(C22V) cargo, together with GFP-Vrg4 to mark the early Golgi and Sec7-HaloTag to mark the late Golgi. A culture was grown to mid-log phase, labeled with JF_{646}, washed, and mounted in a flow chamber. Medium that contained SLF was added while imaging on a confocal microscope, with a Z-stack collected every 3 s. The top panel shows the complete video, with projections of the full Z-stacks. The second panel shows an edited video of two representative cisternae. The bottom three panels show the individual fluorescence channels. Bright-field images of the cells are present in all panels. Scale bar, 2 µm. Frames from this video are shown in Fig. 6 A.

Video 6. **Traffic of cargo to a bleached cisterna during the early-to-late Golgi transition.** Cells expressed the APVNTT-DsRed-Express2-FKBPRD(C22V) cargo, together with GFP-Vrg4 to mark the early Golgi and Sec7-HaloTag to mark the late Golgi. A culture was grown to mid-log phase, treated for 2 h with nocodazole, labeled with JF_{646}, washed, and mounted in a flow chamber. Medium that contained SLF was added for 3 min before imaging on a confocal microscope. The cargo fluorescence in the entire daughter (identified by lack of a nuclear envelope signal) was bleached by illumination with 561-nm laser light at maximum intensity for ∼40 s. Then Z-stacks were captured every 2 s. The video begins shortly before the end of the bleaching period. The top panel shows the complete video, with projections of the full Z-stacks. The second panel shows an edited video of a representative cisterna. The bottom three panels show the individual fluorescence channels. Bright-field images of the cells are present in all panels. Scale bar, 2 µm. Frames from this video are shown in Fig. 7 A.



Video 7. **Departure of cargo during terminal maturation of the late Golgi.** Cells expressed the aggregated APVNTT-DsRed-Express2-FKBPRD(C22V) cargo, together with Sec7-HaloTag to mark the late Golgi and Apl2-GFP to mark AP-1. A mid-log-phase culture was labeled with JF_{646}, then treated with SLF for 5 min before imaging with a confocal microscope. Z-stacks were captured every 1 s. The top panel shows the complete video, with projections of the full Z-stacks. The second panel shows an edited video of two representative cisternae. The bottom three panels show the individual fluorescence channels. Bright-field images of the cell are present in all panels. Scale bar, 2 µm. Frames from this video are shown in Fig. 9.

Provided online is one ZIP file containing annotated SnapGene files for all of the plasmids used in this study.

Reference

Barrero, J.J., E. Papanikou, J.C. Casler, K.J. Day, and B.S. Glick. 2016. An improved reversibly dimerizing mutant of the FK506-binding protein FKBP. *Cell. Logist.* 6:e1204848. https://doi.org/10.1080/21592799.2016.1204848