Supplementary Materials Figure Legends

Figure S1

GalT-CFP and mannosidase II Golgi pool to non-Golgi pool steady state distributions are similar.

A) NRK cells transiently transfected with GalT-CFP were plated on gridded coverslips. Cells in a specific area of the coverslip were imaged. The Golgi and non-Golgi pools of GalT-CFP were quantified by drawing a region of interest around these areas of the cell. Subsequently the cells were fixed and immunostained with primary antibodies against mannosidase II and Cy5-conjugated secondary antibodies. The very same cells on which the GalT-CFP quantifications had been done were re-identified based on their location on the gridded coverslip. The same ROI's were reused for the Golgi and non-Golgi pools of mannosdase II. B) The Golgi and non-Golgi pools of fluorescence for GalT-CFP and mannosidase II were very similar within each cell and the mean Golgi and non-Golgi pools of GalT-CFP and mannosidase II fluorescence from experiments performed as described in part (A) are plotted in the bar graph (n=10 cells).

Figure S2

GalT-CFP and mannosidase II have similar subcellular distribution in mitotic cells

Interphase (A) and prometaphase (B) NRK cells expressing GalT-CFP were fixed and stained with primary antibodies against native mannosidase II and Cy5 conjugated secondary antibodies as described in the methods. Cells were imaged on Zeiss LSM510 with a 63X/1.4NA oil objective, 2 Airy unit pinhole settings and z-slice thickness of 0.5μm. A set of z-sections through a prometaphase cell containing the majority of the mannosidase II labeled fragments is presented in (B).

Figure S3

Native mannosidase II is localized to sec13-YFP labeled ER exit sites in prometaphase stage mitotic cells.

To determine that the GFP tagged Golgi enzyme distribution in prometaphase stage cells was similar to the distribution of native Golgi enzymes, we immunostained stable Sec13-YFP expressing prometaphase stage NRK cells with antibodies to native mannosidase II. The immunostaining was performed as described in the methods. Cy5 conjugated secondary antibodies were used to probe mannosidase II so as not to interfere with the sec13-YFP fluorescence emission. Cells were imaged on Zeiss LSM510 with a 63X/1.4NA oil objective, 2 Airy unit pinhole settings and z-slice thickness of 0.5μm. A set of z-sections through the cell containing the majority of the mannosidase II labeled fragments is presented. Note that almost all mannosidase II labeled fragments are associated with ER exit sites. This result is consistent with our findings with mannosidase II distribution at the ultrastructural level (Figure 2B, mannII-HRP distribution) and the distribution of GalT-CFP at the light level (Figure 2A)

Figure S4 Comparison of Sec13-YFP to native ER exit site protein sec23p.

A**)** NRK cell stably expressing sec13-YFP was fixed in methanol, blocked in 5%FCS/PBS and immunostained first with primary antibodies against the GFP moiety of sec13 and the native ER exit site component sec23p. The cells were then stained with FITC and TRITC tagged secondary antibodies before being mounted and imaged. Note that the overall sec13-YFP and sec23p distributions are nearly identical in the cell.

B) NRK cells (interphase, nocodazole treated and mitotic) were fixed in methanol and stained with antibodies against the native Golgi enzyme mannosidase II and the ER exit site component sec23p as described above. Z stacks (0.5μm) were taken of the interphase, nocodazole treated and mitotic cells. In the nocodazole treated cell many Golgi enzyme-containing fragments are juxtaposed next to ER

exit sites. In the prometaphase mitotic cell there also many mannosidase II containing Golgi fragments juxtaposed to ER exit sites (see arrows in inset). Golgi fragments that appear in this z-slice not to be juxtaposed to an ER exit site (either in the prometaphase cell or the nocodazole treated interphase cell), almost always have an ER exit site in close proximity in the z-plane above or below the one presented in the figure.

Figure S5

Movie of Golgi enzyme and ER exit site dynamics during exit from mitosis.
A single NRK cell stably expressing sec13-YFP was co-transfected with GalT-CFP and imaged using time–lapse confocal microscopy (wide pinhole settings) from metaphase through cytokinesis. Each image was taken 5 seconds apart. Note that ER exit sites are established prior to the emergence of GalT-CFP labeled punctate structures. Moreover practically all GalT-CFP labeled structures emerge from sites in the cytoplasm where there is a pre-existing ER exit site. Once the GalT-CFP fragment has emerged from this

Figure S6

ss-RFP-KDEL is a soluble marker for the endoplasmic reticulum
To demonstrate here that ss-RFP-KDEL is a suitable ER marker we employed a photobleaching assay utilizing fluorescence loss in photobleaching (FLIP). A small region of the ER in cells co-expressing ss-RFP-KDEL and GalT-CFP (as a marker for the Golgi apparatus) was repeatedly photobleached and the loss of fluorescence from surrounding regions of the cell was monitored. As expected ss-RFP-KDEL was exclusively ER-localized (and not additionally localized to pre-Golgi or Golgi structures), since ss-RFP-KDEL fluorescence was quickly lost from the entire cell within 1 minute of repetitive photobleaching. There was no pool that persisted on Golgi or pre-Golgi structures, which because they are discontinuous with ER membranes should have kept any associated fluorescence during the FLIP protocol.

Figure S7

New ts045VSVG-YFP pools are not synthesized or folded during mitosis.

A) To determine that no new ts045VSVG-YFP was being synthesized in the bleached ER pool (Figure 8), we photobleached the entire ts045VSVG-YFP fluorescence in an NRK cell (red outline) about to enter mitosis. Subsequently, time-lapse wide pinhole images collecting fluorescence from the entire depth of the cell were acquired every 1 minute (upper panel) as well as bright field images (taken to monitor the progress of mitosis, lower panel). In addition the fluorescence from the entire cell was quantified (background noise subtracted mean fluorescence per pixel multiplied by total cell area) during the course of mitosis, and plotted in the graph (B). Note that once the ts045VSVG-YFP fluorescence is photobleached there is no recovery of fluorescence. This indicates that there is no new synthesis of ts045VSVG-YFP in mitosis (which is consistent with reports of cessation of protein synthesis in mitosis [Sharff and Robinson 1966]) and moreover no folding of an putative unfolded pool of ts045VSVG-YFP (which would result in the appearance of fluorescence).

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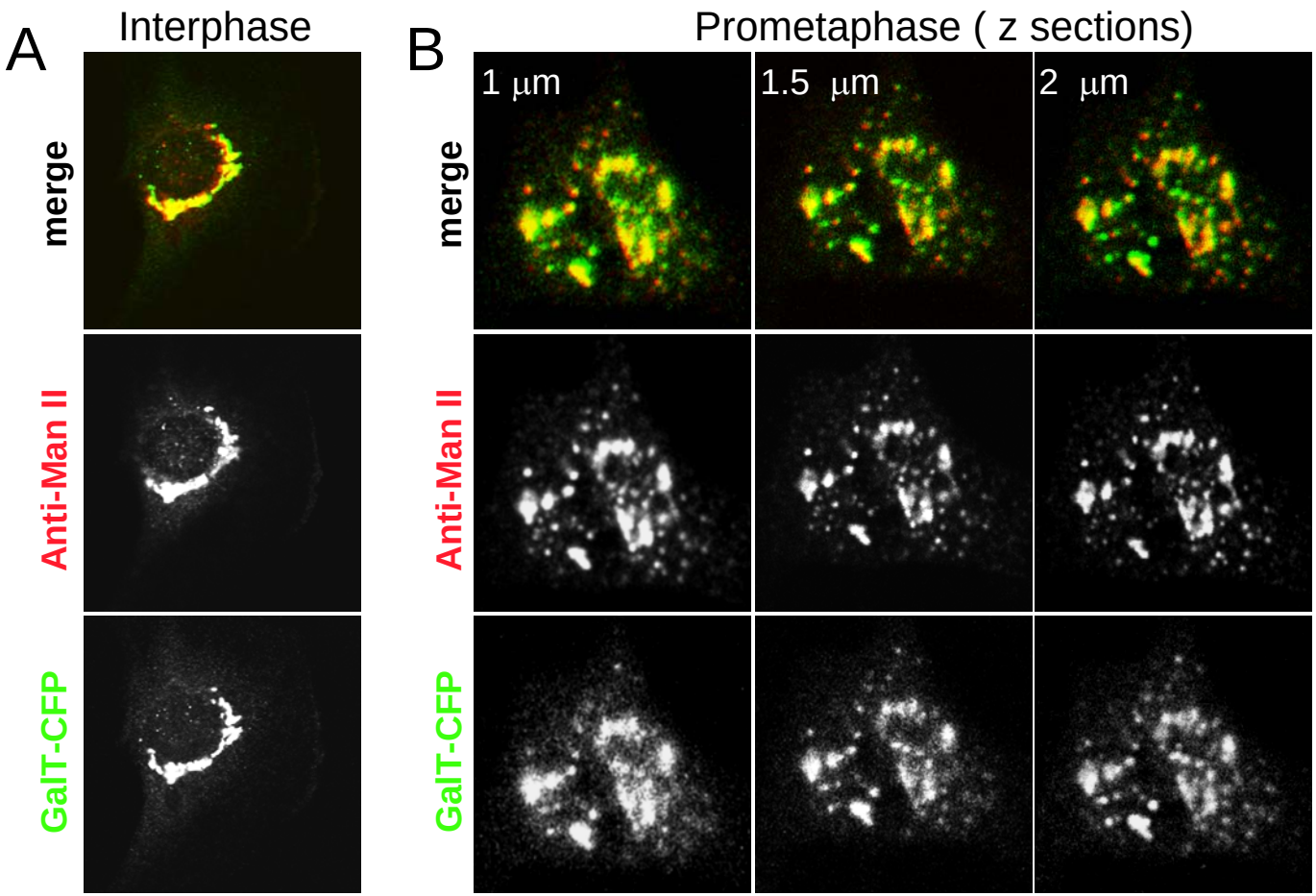
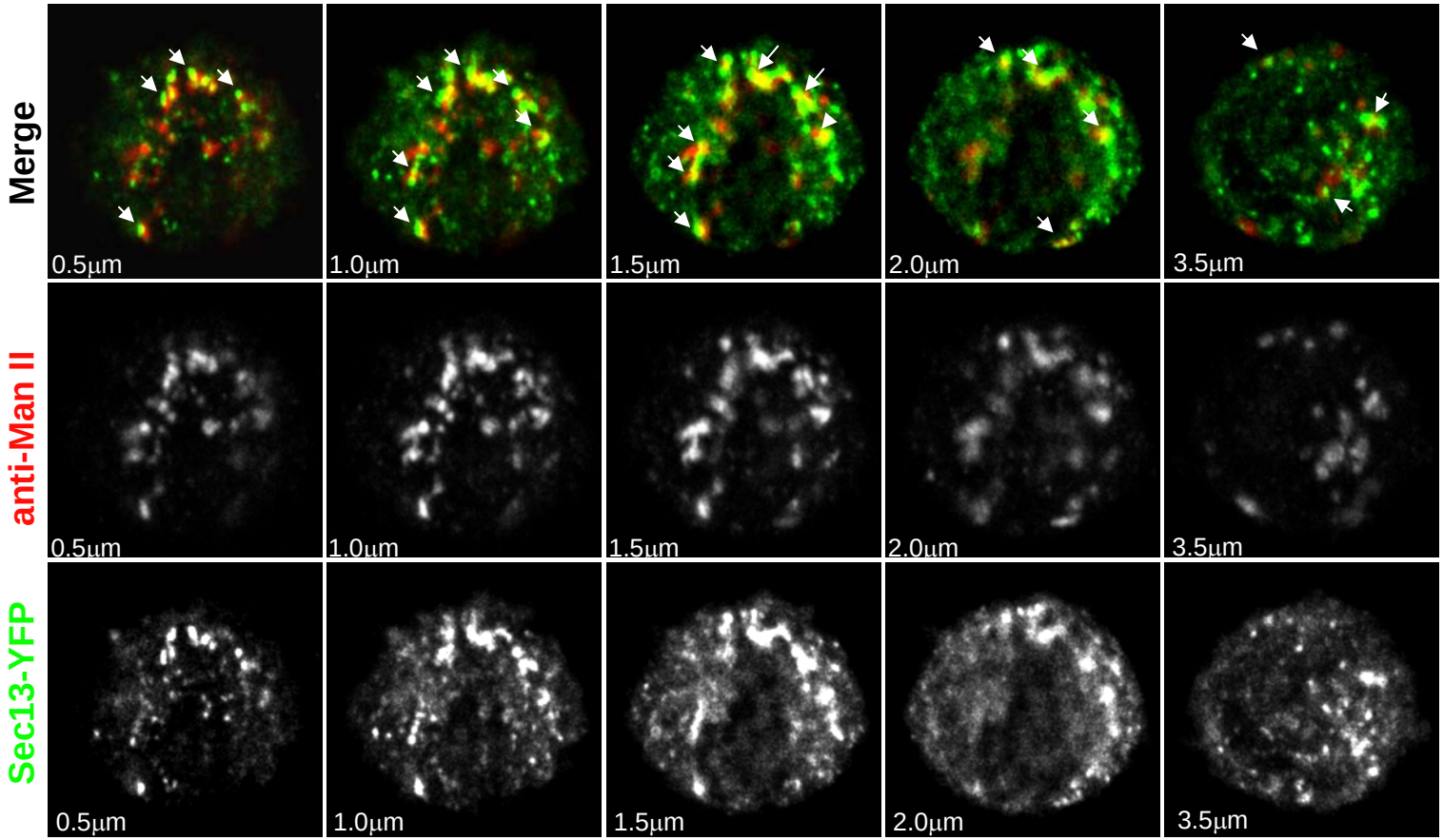
GalT-CFP and native mannosidase II in prometaphase



Altan-Bonnet et al., Figure S2

Z-series in prometaphase cell stably expressing Sec13-YFP and stained with anti-mannosidase II antibodies



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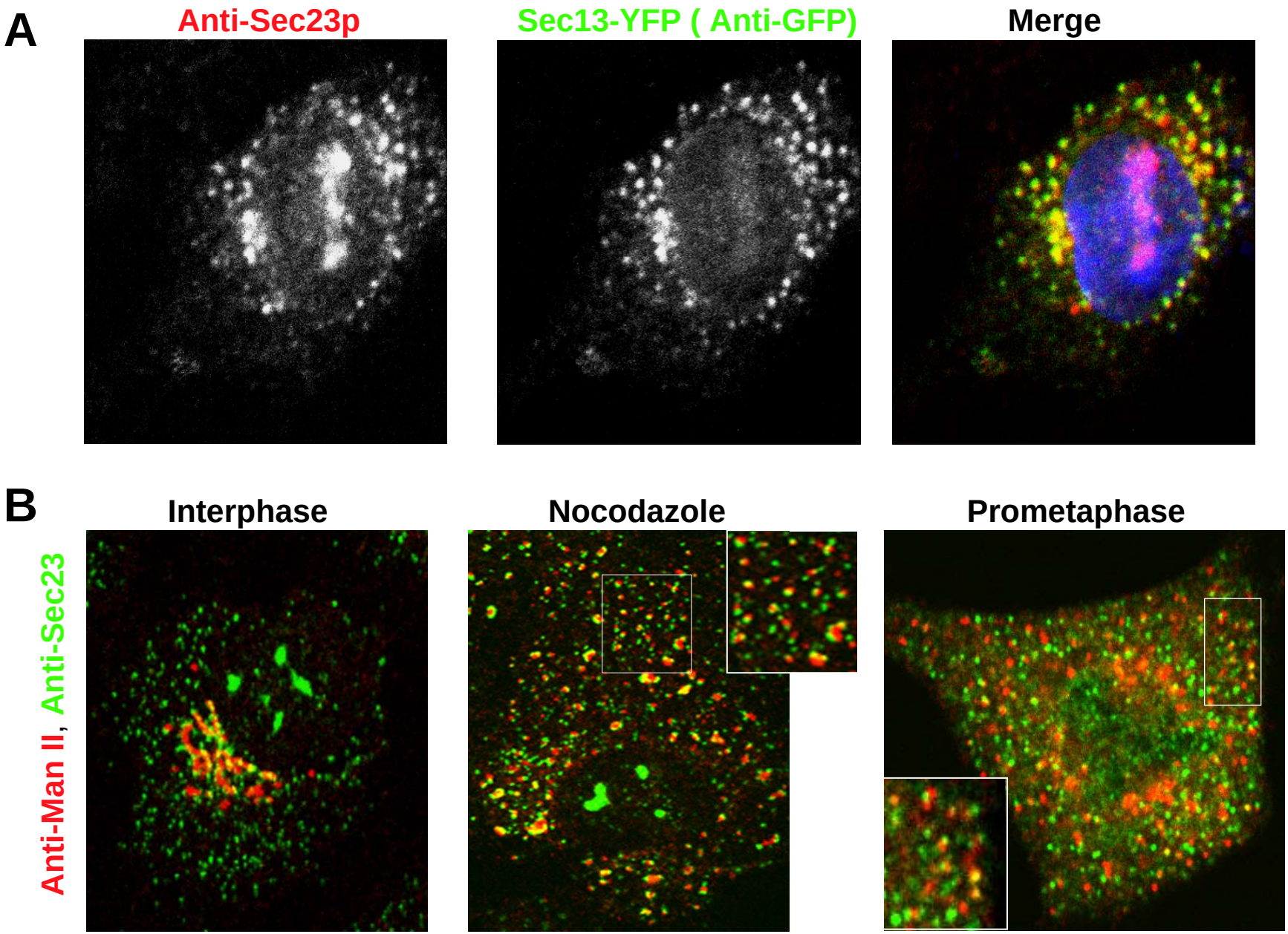
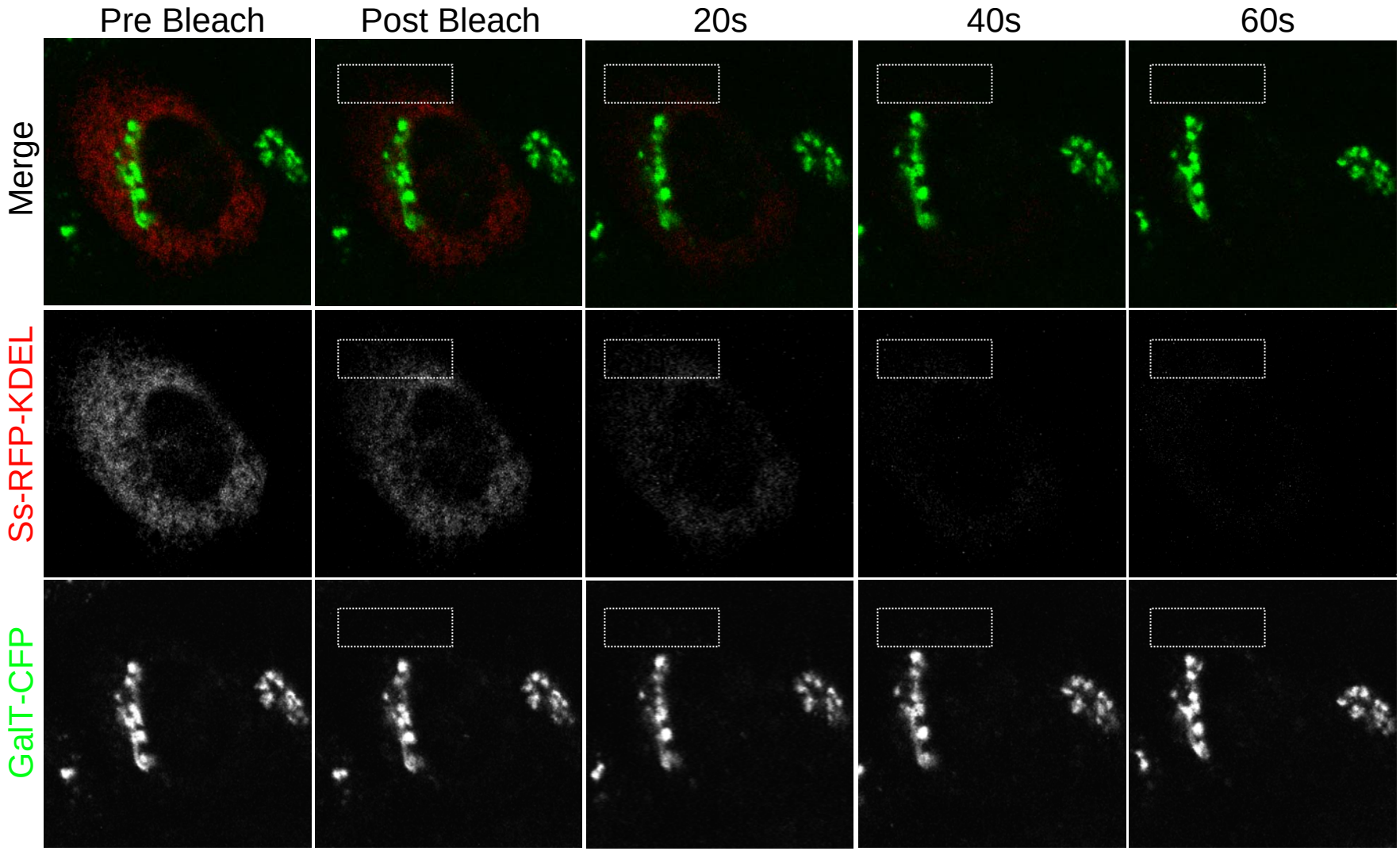
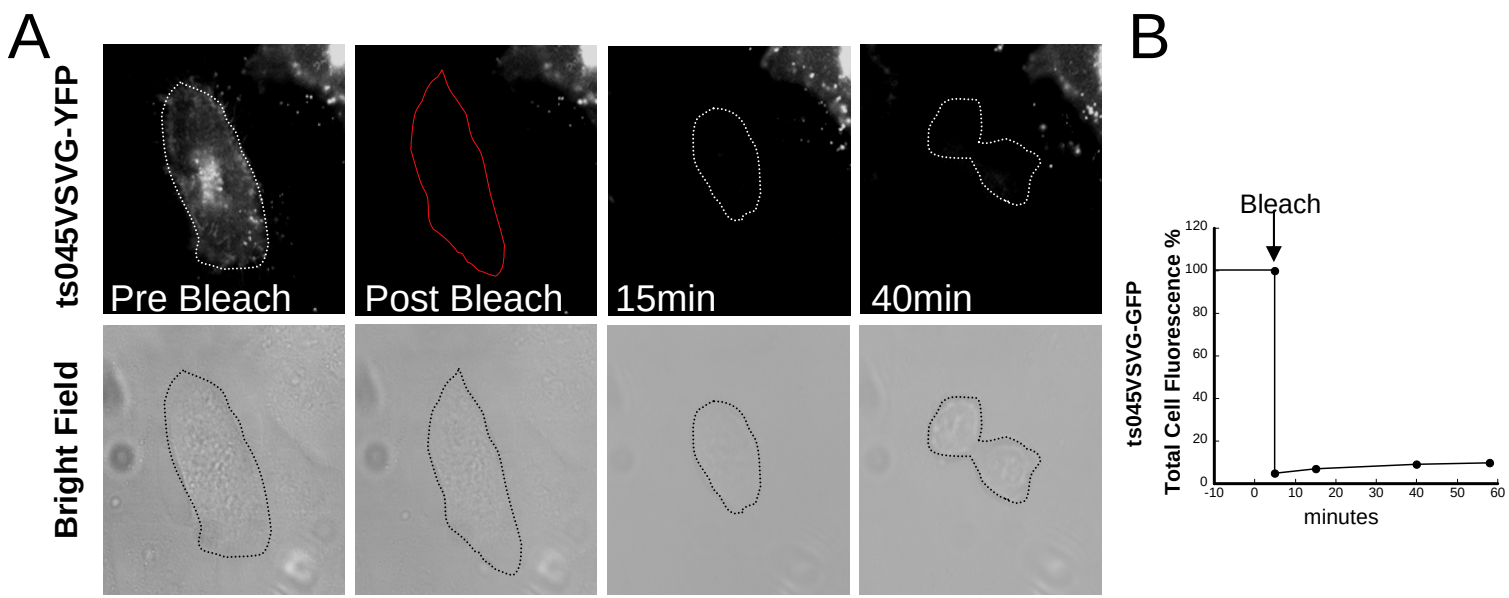


Altan-Bonnet et al., Figure S4

FLIP of ss-RFP-KDEL



Altan-Bonnet et al., Figure S6



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