Supplemental Materials

Molecular Biology of the Cell

Dirck et al.

Dirck et al., Supplemental Figure Legends

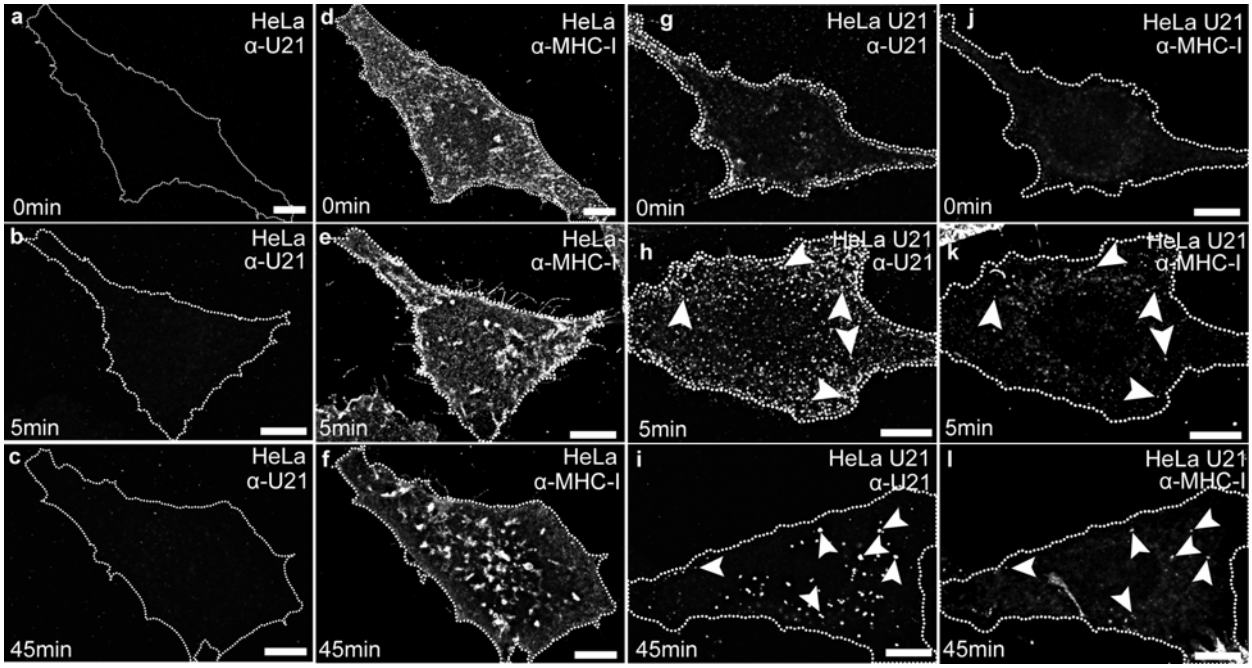

Figure S1. U21 traffics to the plasma membrane. Antibody internalization of α-U21 (MCW62; a-c, g-i) and α-MHC-I (W6/32; d-f, j-l) in HeLa (a-f) and U21-expressing HeLa cells (g-l). To label U21 that reaches the plasma membrane, we performed antibody internalization assays using polyclonal antibodies directed against the extracellular portion of U21 (α-U21N). Cells were incubated with primary antibody for 30 minutes on ice and then warmed to 37°C for the indicated times. The α-U21N antibody bound to U21 on the surface of U21-expressing cells, and did not bind to control HeLa cells (Compare Figure 1b to 1a). After removal of unbound antibody and incubation at 37°C to allow 5 minutes of U21N antibody uptake, peripheral puncta were visible (Figure 1d). After 45 minutes, U21N antibodies were localized to larger, more central perinuclear puncta (Figure 1f). Confocal images are shown. Arrows indicate areas of colocalization for α-U21 and α-MHC-I. Scale bars, 10μm.

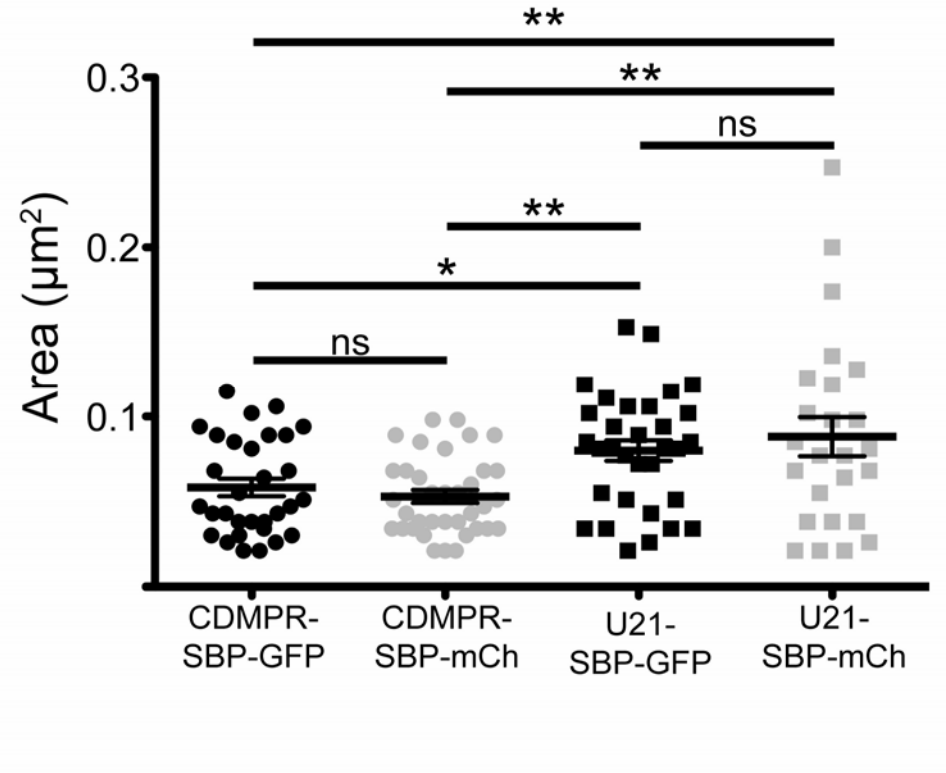

Figure S2. RUSH U21-containing vesicles are larger than RUSH CD-MPR-containing vesicles irrespective of the fluorescent protein tag. HeLa cells expressing U21-SBP-mCherry (gray squares), U21-SBP-GFP (black squares), SBP-mCherry-CDMPR (gray circles), and SBP-GFP-CDMPR (black circles) reporter proteins were incubated with biotin for 45-60 minutes to synchronously release the reporter proteins from the ER. Cells were fixed and images were acquired on a structured illumination microscope. The area was determined for vesicles containing SBP-GFP-CDMPR (n = 31), SBP-mCherry-CDMPR (n = 36), U21-SBP-GFP (n = 32), and U21-SBP-mCherry (n = 25). Vesicle sizes were compared by one-way ANOVA analysis with a Newman-Keuls Multiple Comparison test applied to show significance. ns = not significant; *, $p < 0.05$; **, $p < 0.01$.

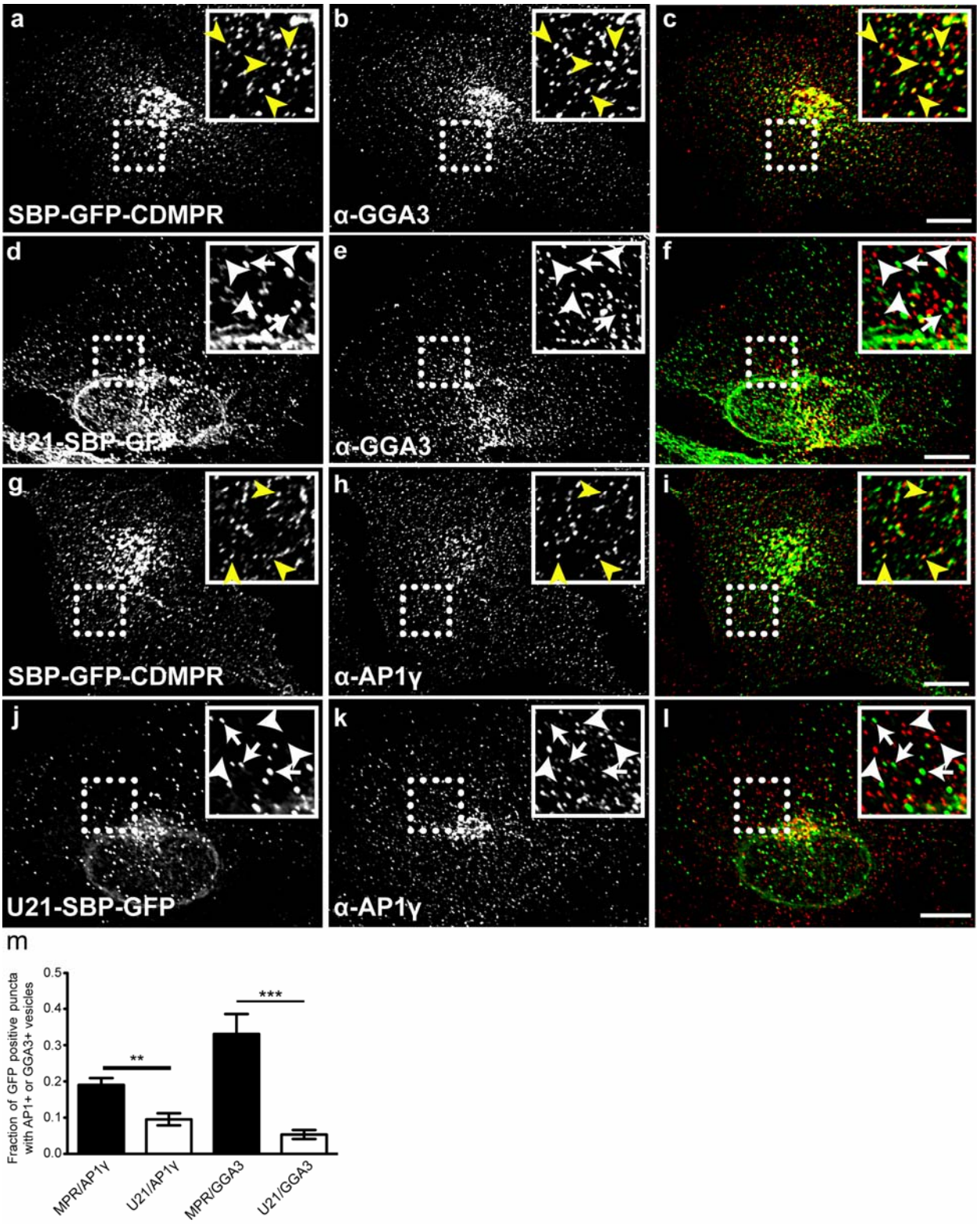

Figure S3. U21 does not colocalize with AP1 or GGA3 clathrin-adaptor proteins. a-l) Confocal colocalization of CDMPR or U21 with GGA3 (a-f) or AP-1γ (g-l) in HeLa cells expressing either RUSH SBP-GFP-CDMPR (a-c, g-i) or U21-SBP-GFP (d-f, j-l). Cells were incubated

with biotin for 60 minutes and then fixed and labeled with antibody directed against GGA3 (a-f) or AP-1γ (g-l). Enlarged views are depicted in boxed regions. a-c) Yellow arrowheads indicate points of colocalization between SBP-GFP-CDMPR (green) and GGA3 (red). d-f) Arrows indicate U21-SBP-GFP -positive puncta. Arrowheads indicate GGA3-positive puncta. g-i) Yellow arrowheads indicate points of colocalization between SBP-GFP-CDMPR (green) and AP-1γ (red). j-l). Arrows indicate U21-SBP-GFP-positive puncta. Arrowheads indicate AP-1γ-positive puncta. m) Percentage of CDMPR-containing vesicles that colocalize with AP-1γ or GGA3, as noted (black bars), and percentage of U21-containing vesicles that colocalize with AP-1γ or GGA3, as noted (white bars). Three 10x10μm boxes per cell were counted for each biological replicate, for a total of 9 replicates per sample. The fraction of GFP-positive puncta per AP-1γ-positive or GGA3-positive puncta are shown as mean ± SEM. The number of AP-1γ or GGA3 vesicles per replicate ranged between 11 and 40, with a mean of ~29 and median of 31. Scale bars, 10μm. **, p = 0.0016; ***, p = 0.0002.

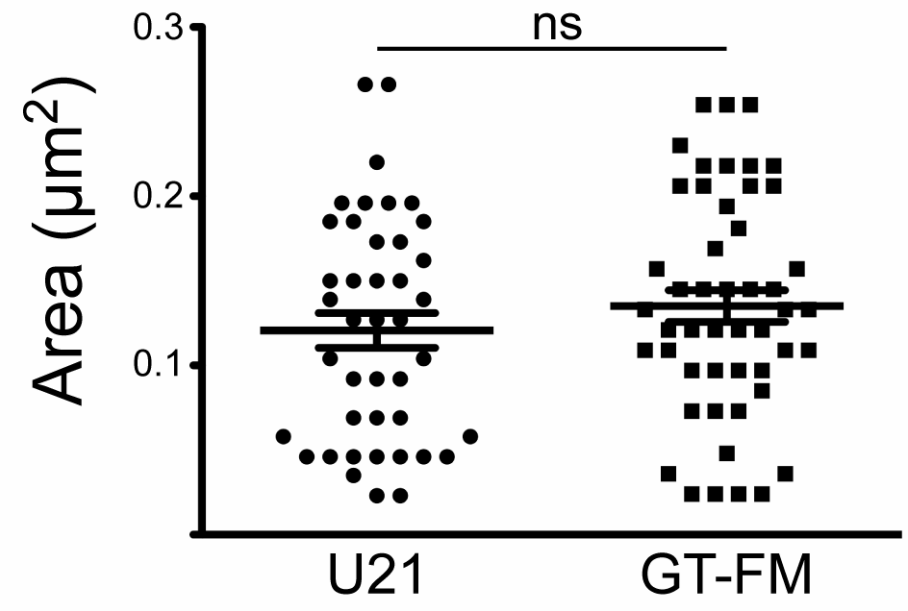

Figure S4. GT-FM-containing vesicles are similar in size to U21-containing vesicles. Structured illumination microscopy of HeLa cells expressing either U21-SBP-GFP (circles) or GalT-3xFM-GFP (GT-FM; squares). U21-SBP-GFP-expressing HeLa cells were incubated with biotin for 60 minutes. GT-FM-expressing HeLa cells were incubated for 60 minutes in the absence of D/D solubilizer, and fixed. 8-10 cells for U21-SBP-GFP or GT-FM-GFP were analyzed for vesicle size. The number of vesicles counted per cell ranged from 3 to 7, with a mean of 5 and median of 5. Data is shown as mean ± SEM. U21-SBP-GFP: 0.1207μm^2 (n = 41). GT-FM: 0.1351 μm^2 (n = 49). p = 0.3034.