Rizzo et al., http://www.jcb.org/cgi/content/full/jcb.201211147/DC1

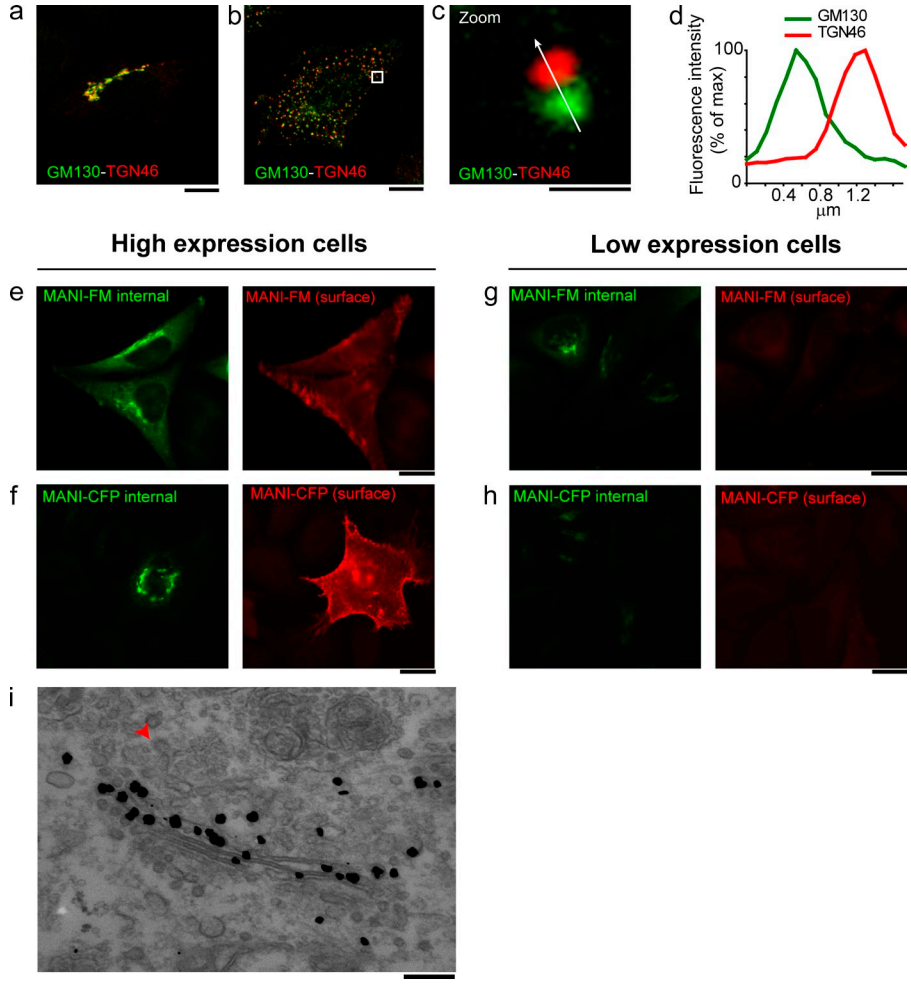


Figure S1. **FM fusion proteins localize mainly to the Golgi and have appropriate sub-Golgi localizations.** (a) Untreated HeLa cells show a typical ribbon-like structure of the Golgi. GM130 staining is in green and TGN46 in red. (b) Nocodazole treatment (33 µM for 3 h) fragments the intact Golgi ribbon to hundreds of ministacks spread throughout the cell. GM130 staining is in green and TGN46 in red. (c) Ministacks resulting from nocodazole treatment maintain polarity as seen by a clear separation of GM130 (green) and TGN46 (red). A magnified image of a single isolated stack from b. White arrow across the stack was used for line-scan analyses. (d) Fluorescence intensity distribution of markers along the line scan (c, arrow). The fluorescence intensities were normalized to their respective peak values. The image shown is representative of more than three independent experiments and, for the experiment shown, $n = 10$. (e–h) Monomeric MANI-FM is mainly retained in the Golgi. HeLa cells expressing high or low levels of MANI-FM (+AP; e and g) or MANI-CFP (f and h) were fixed and labeled for surface localized MANI-FM and MANI-CFP using anti-HA (monoclonal antibody) and anti-GFP antibodies, respectively, without permeabilization (red). Later, cells were permeabilized and labeled for proteins present internally (green; anti-HA polyclonal for MANI-FM; MANI-CFP was not labeled). In cells expressing high levels of the proteins, both MANI-FM and MANI-CFP are present in the PM. (i) HeLa cells transfected with GALT-FM in the presence of AP were fixed and stained for GALT-FM (anti-HA) using immunonanogold. The trans side of the stack was identified by clathrin-coated structures (arrowhead). The labeling is mainly present in the trans-most cisterna of the stack. Bars: (a and b) 15 µm; (c) 1 µm; (e) 18 µm; (f) 16 µm; (g and h) 20 µm; (i) 150 nm.

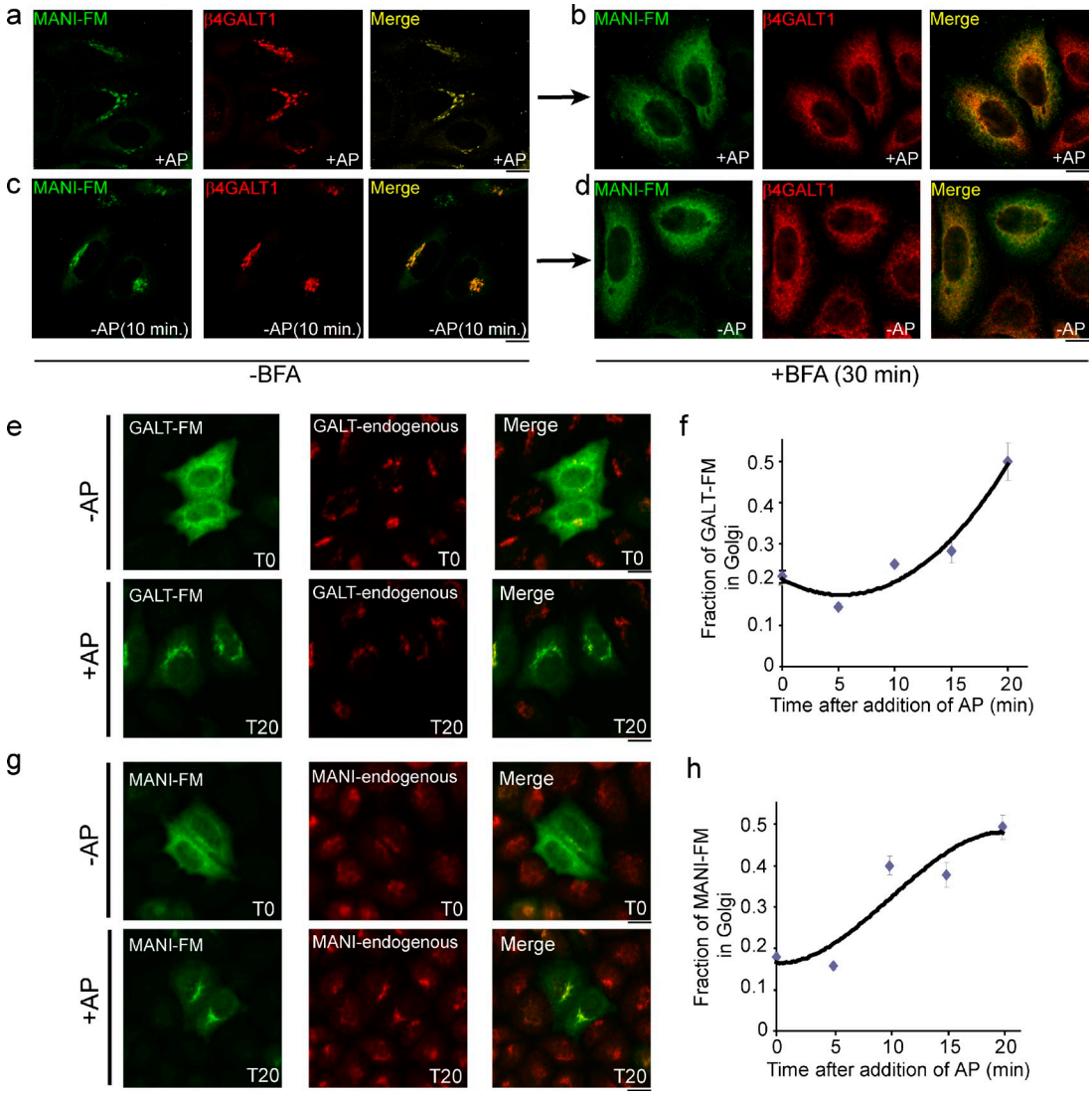


Figure S2. **Polymerization of FM fusion proteins does not prevent their BFA-induced retrograde transport to ER but prevents their exit out of the ER.** HeLa cells expressing MANI-FM cultured in the continued presence of AP (a and b) or in the absence of AP for 10 min (c and d) were incubated with BFA for 30 min (b and d) in the presence or absence of AP, respectively, and the localization of the transfected proteins visualized by IF. BFA treatment induces the ER translocation of both monomeric and polymeric MANI-FM. (e–h) Polymerization of FM fusion proteins prevents their exit out of the ER. (e) GALT-FM is retained in the ER in the absence of AP (−AP) and is rapidly transported to the Golgi after addition of AP (+AP). (f) Time kinetics of GALT-FM transport to the Golgi after addition of AP. The curve fitting was done using a third order polynomial function. (g) Similar to e, MANI-FM transport was monitored. (h) Similar to f, time kinetics of MANI-FM transport to Golgi. Antibodies against the endogenous GALT (e) and MANI (g) were used to identify the Golgi apparatus. About 20–30 cells per time point were analyzed. Values are mean ± SEM. Bars: (a and b) 16 µm; (e and g)18 µm.

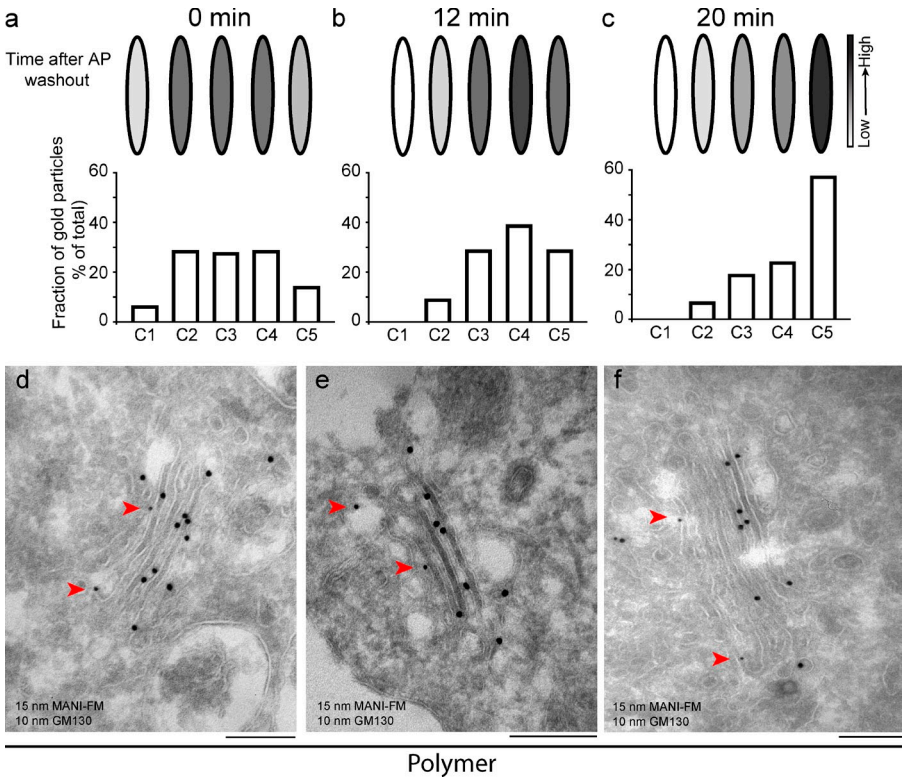
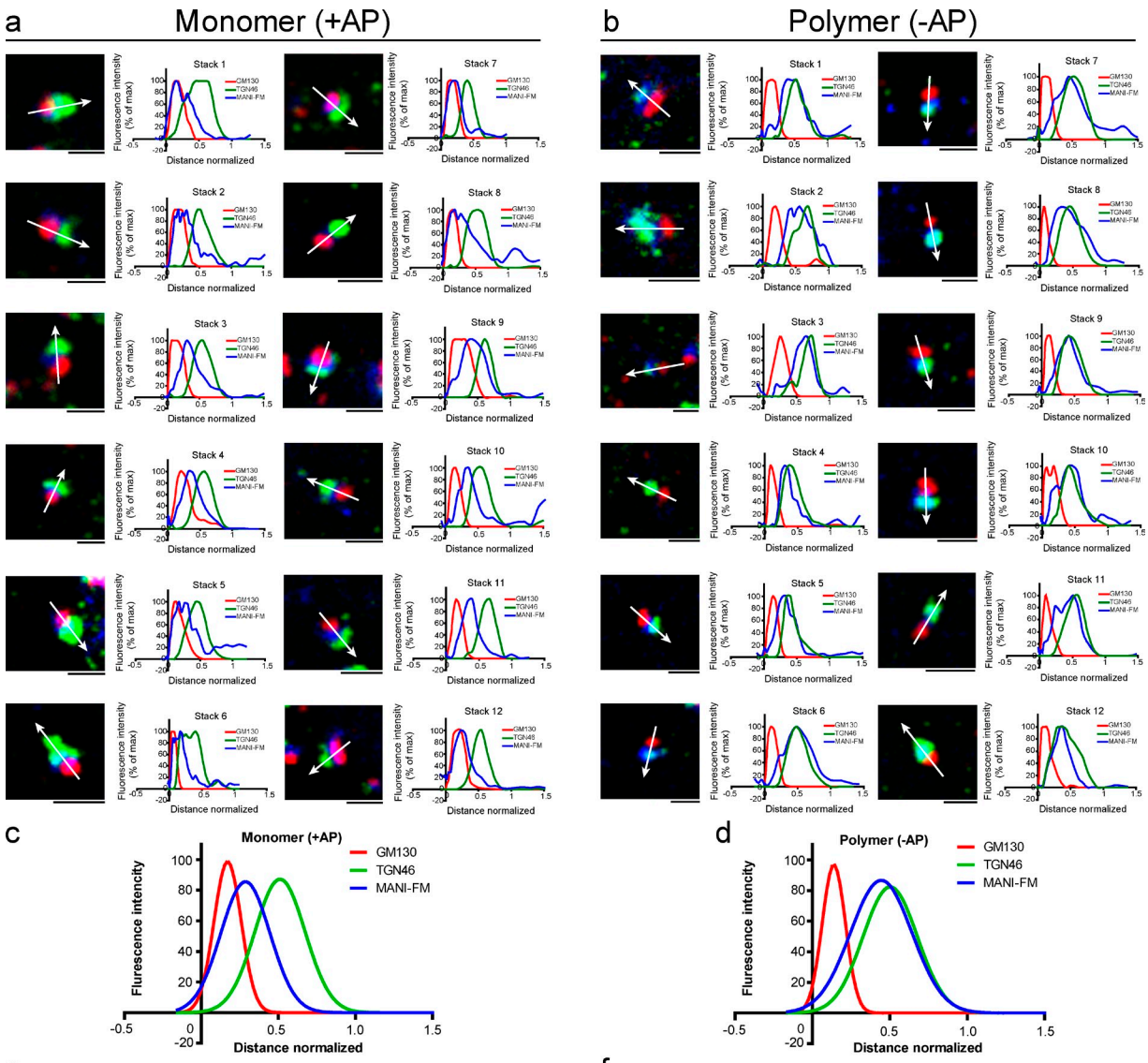


Figure S3. **Frequency distribution analysis and double immunolabeling with GM130 as cis marker confirms that polymerization of MANI-FM induces its shift from cis to trans side.** HeLa cells expressing MANI-FM cultured in the presence of AP (a) or after AP washout for 12 (b) or 20 (c) min were fixed and processed for cryoimmunogold labeling. The trans-most cisterna (trans side identified by the presence of clathrin coats) was named as C5 and the rest of the cisterna assigned accordingly, with C1 being the fifth cis-most cisterna. The frequency distribution of the gold particles across the cisterna was quantified and represented as fraction of gold particles present in each cisterna. Notably, 40% of the stacks had three cisternae, 50% had four cisternae, and only 10% of the stacks had five. Given the procedure used for the calculations, this accounts for the low values of gold particles in the cisternae C1 and C2. Nevertheless, the shift of the MANI-FM peak from the cis/medial to the trans side of the stack remains evident, as shown in the scheme above the graph. The intensity of the filling color corresponds to the number of gold particles of MANI-FM (see intensity scale on the right). At least 20 Golgi profiles per time point were used for quantitation and >100 gold particles per time point were counted from two independent experiments. For this experiment, $n > 20$ for each time point. (d–f) HeLa cells expressing MANI-FM were cultured in the presence of AP and then AP was washed out for 20 min before fixing and processing for cryoimmunogold labeling. The cis side of the stack was identified by GM130 (arrowheads; 10-nm gold). Polymerization leads to the translocation of the MANI-FM (15-nm gold) to the trans side. Bars, 180 nm.



e Monomer (+AP)

	GM130	TGN46	MANI-FM
Gaussian			
Best-fit values			
Amplitude	98.47	87.07	85.44
Mean	0.1705	0.5106	0.2860
SD	0.08846	0.1637	0.1599
Std. Error			
Amplitude	3.020	2.892	2.760
Mean	0.003120	0.006297	0.005881
SD	0.002833	0.006047	0.005468
95% Confidence Intervals			
Amplitude	92.55 to 104.4	81.40 to 92.73	80.04 to 90.85
Mean	0.1643 to 0.1766	0.4983 to 0.5230	0.2745 to 0.2975
SD	0.08291 to 0.09401	0.1519 to 0.1756	0.1492 to 0.1706
Goodness of Fit			
Degrees of Freedom	351	351	351
R square	0.7509	0.7197	0.6455
Absolute Sum of Squares	113798	109618	132419
Sy.x	18.01	17.67	19.42
Constraints			
SD	SD > 0.0	SD > 0.0	SD > 0.0
Number of points			
Analyzed	354	354	354

f Polymer (-AP)

	GM130	TGN46	MAN1-FM
Gaussian			
Best-fit values			
Amplitude	96.77	82.33	86.61
Mean	0.1386	0.5025	0.4446
SD	0.07518	0.1755	0.1986
Std. Error			
Amplitude	3.068	2.706	2.520
Mean	0.002723	0.006675	0.006683
SD	0.002470	0.006368	0.006088
95% Confidence Intervals			
Amplitude	90.75 to 102.8	77.02 to 87.63	81.67 to 91.55
Mean	0.1332 to 0.1439	0.4894 to 0.5156	0.4315 to 0.4577
SD	0.07034 to 0.08002	0.1630 to 0.1880	0.1867 to 0.2105
Goodness of Fit			
Degrees of Freedom	322	322	322
R square	0.7681	0.7419	0.7344
Absolute Sum of Squares	82667	84112	89134
Sy.x	16.02	16.16	16.64
Constraints			
SD	SD > 0.0	SD > 0.0	SD > 0.0
Number of points			
Analyzed	325	325	325

Figure S4. **Shift of polymeric MANI-FM from cis- to trans-Golgi as visualized by confocal microscopy.** HeLa cells expressing MANI-FM cultured in the presence of AP (a) or AP washed for 20 min (b) were fixed and processed for IF labeling. The white arrows across the stacks were used for line-scan analyses. Representative stacks from the same pool of images from which Fig. 4 (f and g) was selected are shown. (c–f) Computational coalescence of the 12 graphs shown in a and b are shown in c and d, respectively, with the associated parameters used for curve fitting in e and f. This is a representative experiment out of three independent experiments. For this experiment, *n* = 12. Bars, 1 µm.

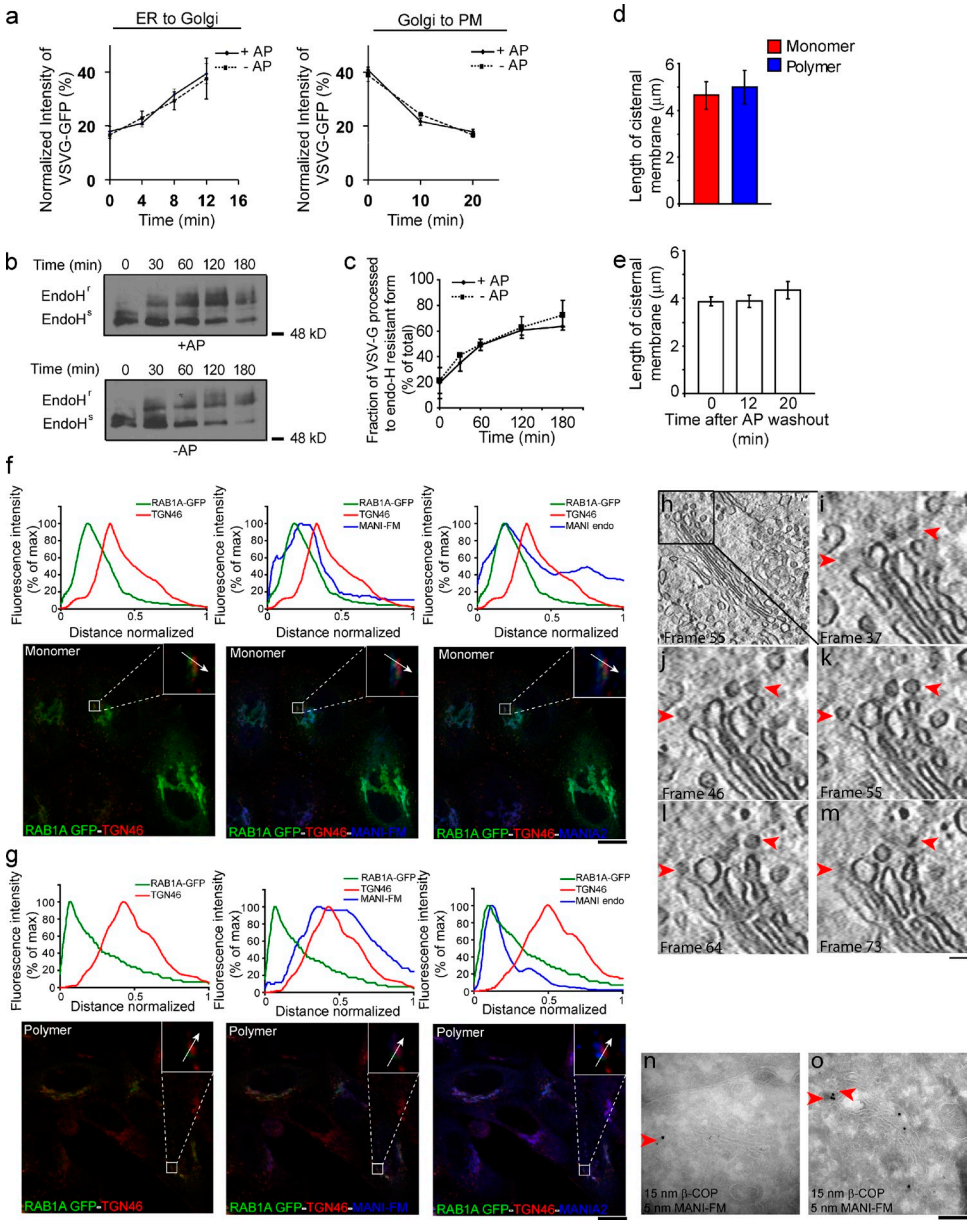


Figure S5. **Polymerization of MANI-FM does not affect the morphology or the functioning of the Golgi.** (a) Transport of VSV-G-GFP from ER (cells maintained at 40°C) to Golgi (after shift to 32°C for the indicated times) or transport out of the Golgi (shift back to 40°C for indicated times after 20 min at 32°C to accumulate the protein in the Golgi apparatus), quantitated by measuring the fluorescence intensity of VSV-G-GFP at the Golgi region, was not affected by the polymeric state of MANI-FM (+AP, monomer; –AP, polymer). The fluorescence intensity of the VSV-G-GFP at the Golgi was normalized to its total intensity in the cell. The assay was done with intact Golgi ribbon and nocodazole was not included in these experiments. At least 20 cells per time point per experiment were analyzed. Values are mean ± SEM. (b) VSV-G glycosylation as measured by acquisition of resistance to EndoH (EndoH^r-EndoH resistant and EndoH^s-EndoH sensitive) was not affected by the polymeric state of MANI-FM (+AP, monomer; –AP, polymer). Assay was again done using intact Golgi ribbon in the absence of nocodazole. (c) Quantitation of the acquisition of EndoH resistance represented as the fraction of VSV-G that was resistant to digestion by Endoglycosidase H. Values are mean ± SEM, from three different experiments. (d and e) Morphology of the Golgi stacks is not affected by MANI-FM polymerization. HeLa cells expressing MANI-FM cultured in the presence of AP (+AP) or AP washed out for 20 min (−AP), in the absence (d) or presence (e) of nocodazole, were fixed and processed by EM imaging. The length of the Golgi cisternal membranes were measured and expressed in micrometers. The cisternal membrane length (as a measure of surface area) did not change appreciably after MANI-FM polymerization. (f and g) MANI-FM polymerization does not change the localization of endogenous Mannosidase I. HeLa cells cotransfected with MANI-FM and Rab1A-GFP were cultured in the presence of AP (f) and AP was washed out for 20 min (g) before fixing and processing for IF labeling. The experiment was done in the absence of nocodazole to maintain the intact Golgi ribbon. The white arrow across the ribbon was used for line-scan analyses shown above the images. The fluorescent intensities were normalized to their respective peak values. The images shown are representative of at least 10 Golgi profiles examined for each condition from two independent experiments. For the experiment shown, $n = 10$. The Polymerization shifts MANI-FM (blue) from the cis (Rab1-GFP, green) to trans side (TGN46, red), whereas the endogenous MANI (MANI endo, blue) maintains its cis/medial position. (h–m) Tomography reconstruction shows that round profiles seen in thin sections are indeed vesicles. A single frame from the tomographic reconstruction of a Golgi stack (h). The boxed area containing round profiles is enlarged in i–m. Shown are frames from the tomographic reconstruction where two round profiles (arrowheads) were followed through, to validate them as isolated vesicles. (n and o) HeLa cells expressing MANI-FM were cultured in the presence of AP and then AP was washed out for 20 min before adding it back again for 1.5 min. The cells were then fixed and processed for cryoimmunogold labeling with anti–β-COP antibodies (15-nm gold) and anti-HA antibodies (5-nm gold; MANI-FM). The COPI-coated structures contain MANI-FM (arrowheads). Bars: (f and g) 20 µm; (h) 180 nm; (i–m) 60 nm; (n) 200 nm; (o) 180 nm.