Supplementary Materials for

The specificity of vesicle traffic to the Golgi is encoded in the golgin coiled-coil proteins

Mie Wong and Sean Munro

correspondence to: sean@mrc-lmb.cam.ac.uk

This PDF file includes:

Figs. S1 to S16
Captions for Movies S1 and S2

Other Supplementary Materials for this manuscript includes the following:

Movies S1 and S2

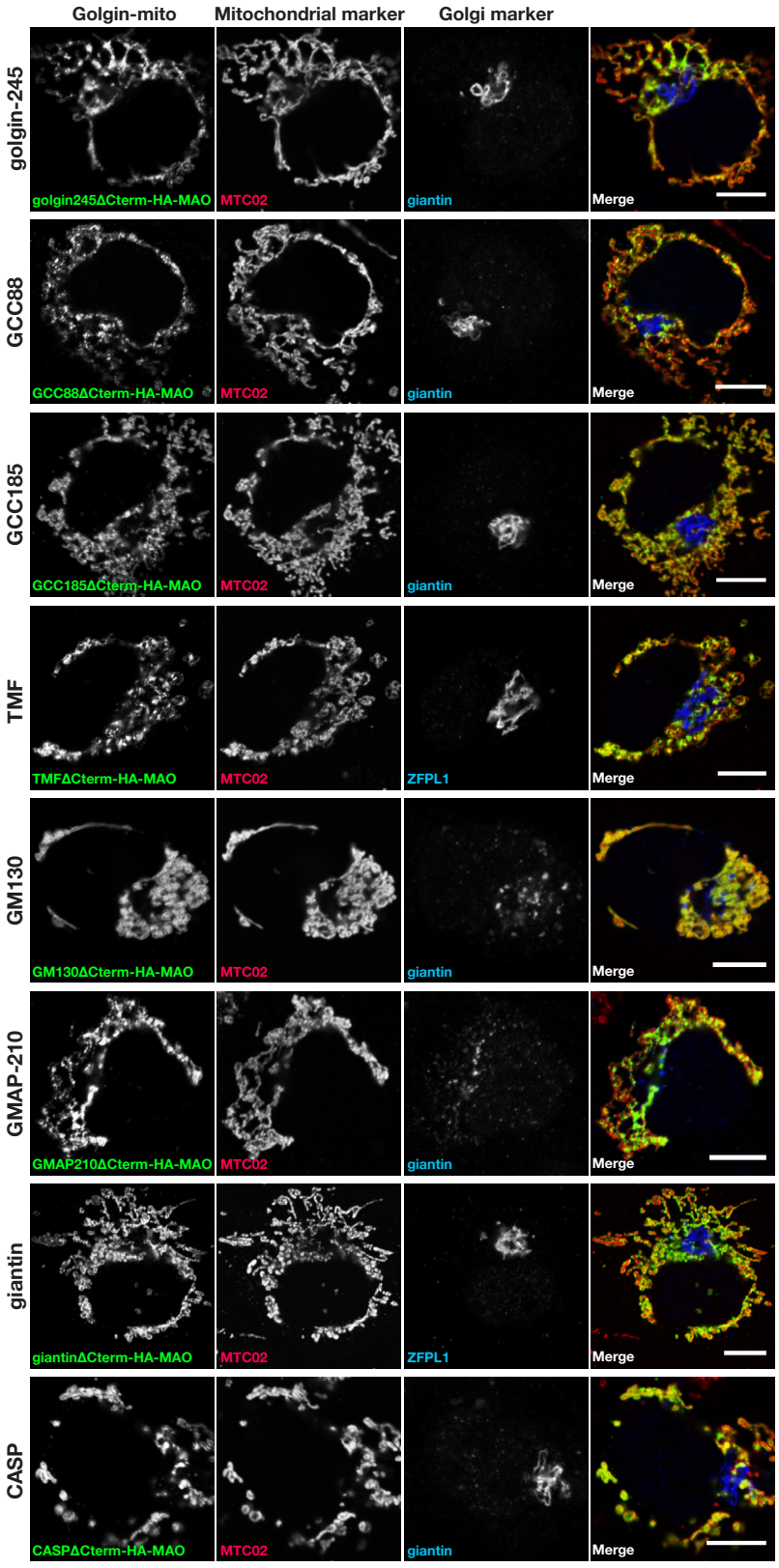

Fig. S1. Ectopic location of golgins on mitochondria.
Confocal micrographs of COS cells expressing those golgin-mito fusion proteins not shown in Fig. 1F, and stained for the HA epitope in the fusion. Costaining for markers for the mitochondria (MTC02) and Golgi (ZFPL1 (cis) or giantin (trans)) reveal targeting of each of the golgins to mitochondria. Scale bars 10 μm.

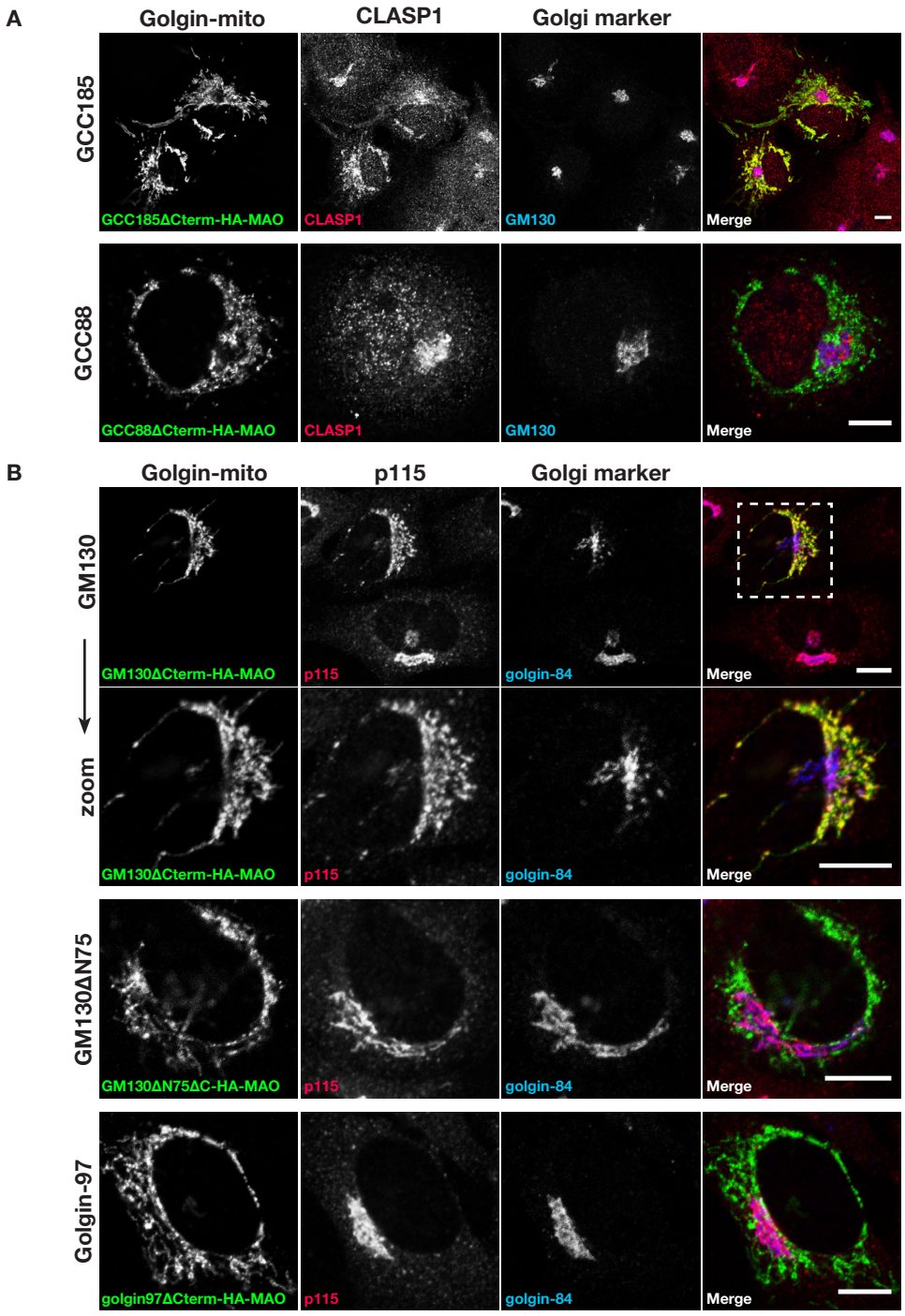

Fig. S2. Relocation of known golgin binding partners.
(**A**) Confocal images of COS cells expressing the indicated golgin-mito and co stained for either CLASP1 (a reported GCC185 interactor (*14*)), or the cis Golgi marker, GM130. Scale bars 10 µm. (**B**) MEFs expressing the indicated golgin-mito were costained for p115 (a reported interaction partner for GM130 (18)); and a Golgi marker, golgin-84 (medial). Scale bars 10 µm.

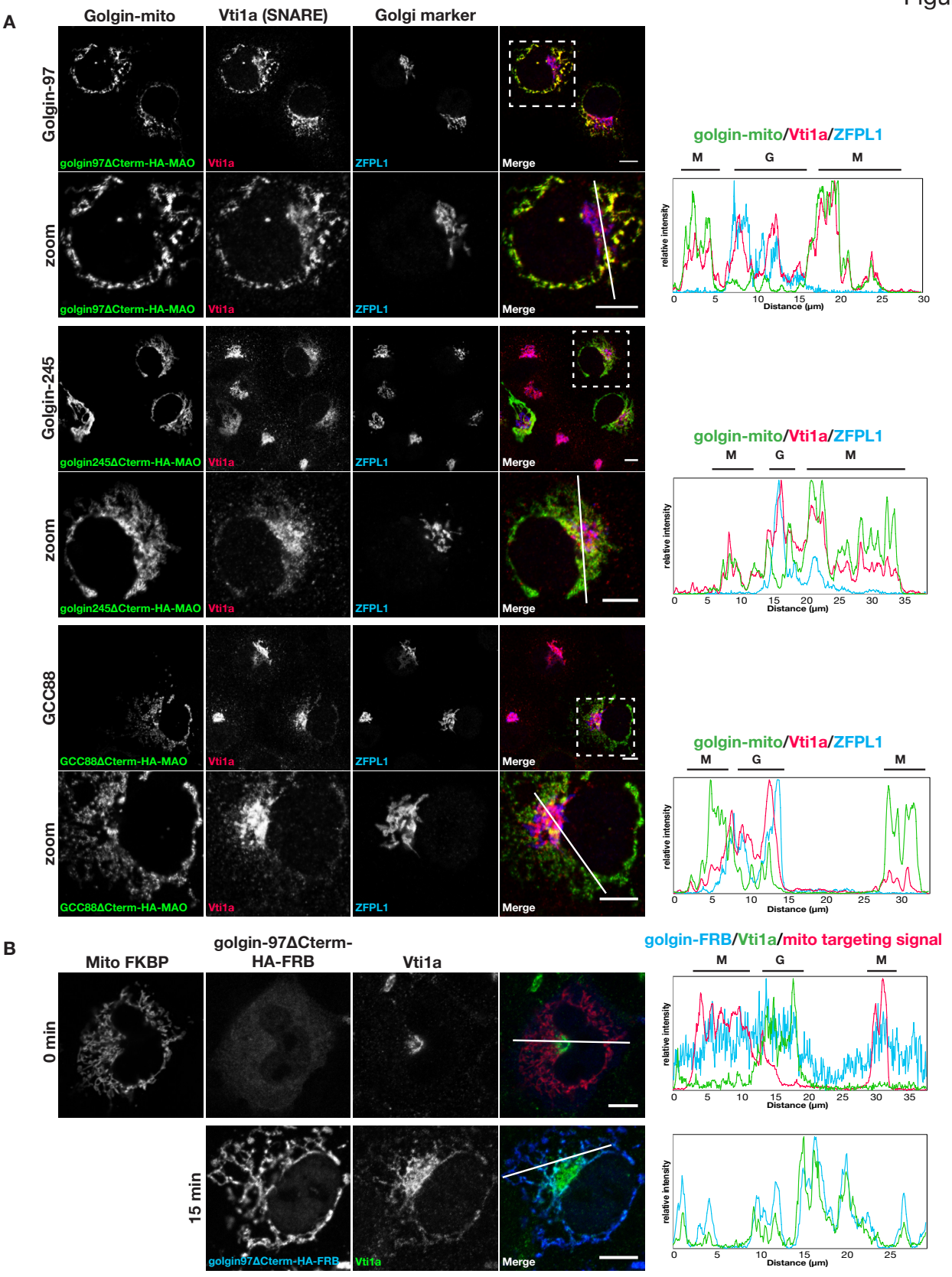

Fig. S3. Golgin-97, golgin-245 and GCC88 relocate the endosome-to-TGN SNARE Vti1a.
(**A**) Confocal micrographs of COS cells expressing the indicated golgin-mito (HA) were costained for endogenous Vti1a and a Golgi marker (ZFPL1). Plots of relative signal intensity against distance show overlap between channels. The location of mitochondria (M) and the Golgi (G) are indicated. Scale bars 10 μm. (**B**) COS cells coexpressing golgin-97-HA-FRB and FKBP-mCherry-MAO were treated with 200 nm rapamycin, fixed at the indicated time points and stained for endogenous Vti1a. Plots and scale bars as in (A).

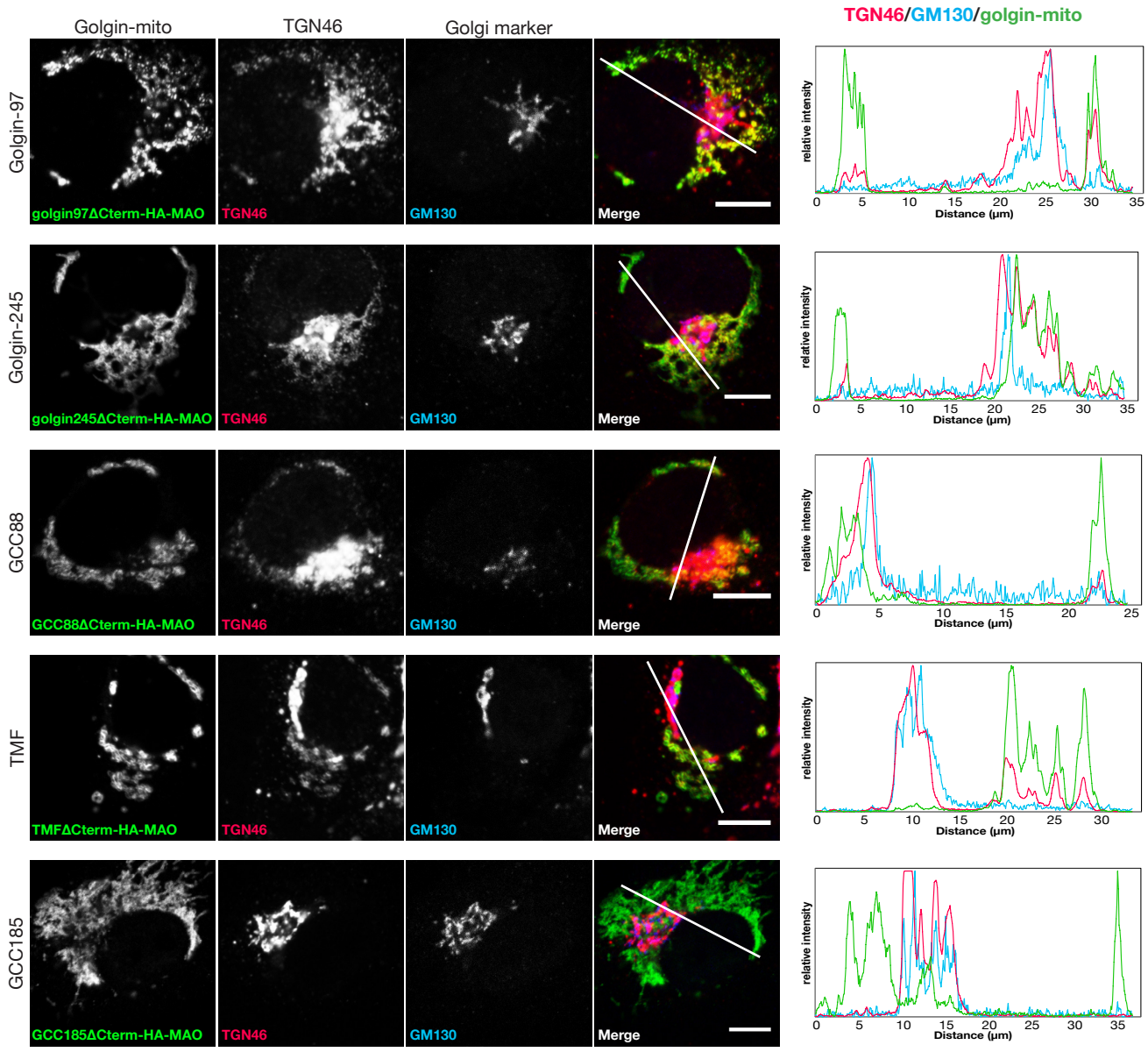

Fig. S4. Golgin-97, golgin-245, GCC88 and TMF relocate TGN46 to mitochondria.
Confocal micrographs of COS cells expressing the indicated golgin-mito (HA) were costained for endogenous TGN46 and the Golgi-marker GM130 (cis). Plots of relative signal intensity against distance show occurrence of overlap between channels. Scale bars 10 μm.

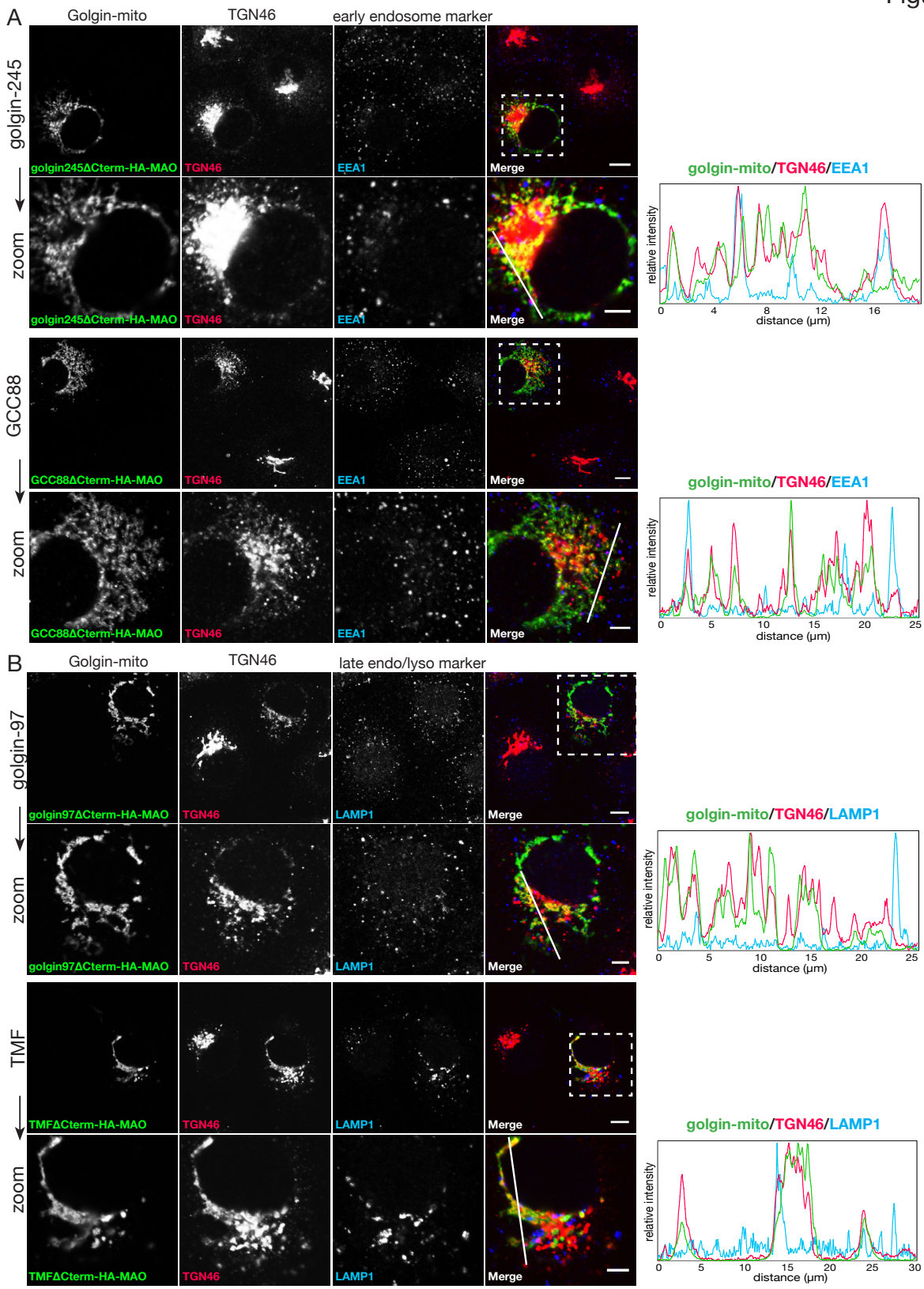

Fig. S5. Golgin-97, golgin-245, GCC88 and TMF relocate endosome-to-TGN cargo, but not endosomes. (**A**) Confocal images of COS cells expressing the indicated golgin-mito (HA) were costained for endogenous TGN46 (endosome to TGN cargo) and EEA1 (early endosomes). Plots of relative signal intensity against distance show occurrence of overlap between channels. Scale bars 10 μm or 5 μm (zoom). Golgin-97 and TMF showed a similar lack of overlap with EEA1. (**B**) as (A), except that staining was for endogenous LAMP1 (late endosomes and lysosomes). Intensity plots and scale bars as in (A). Golgin-245 and GCC88 showed a similar lack of overlap with LAMP1.

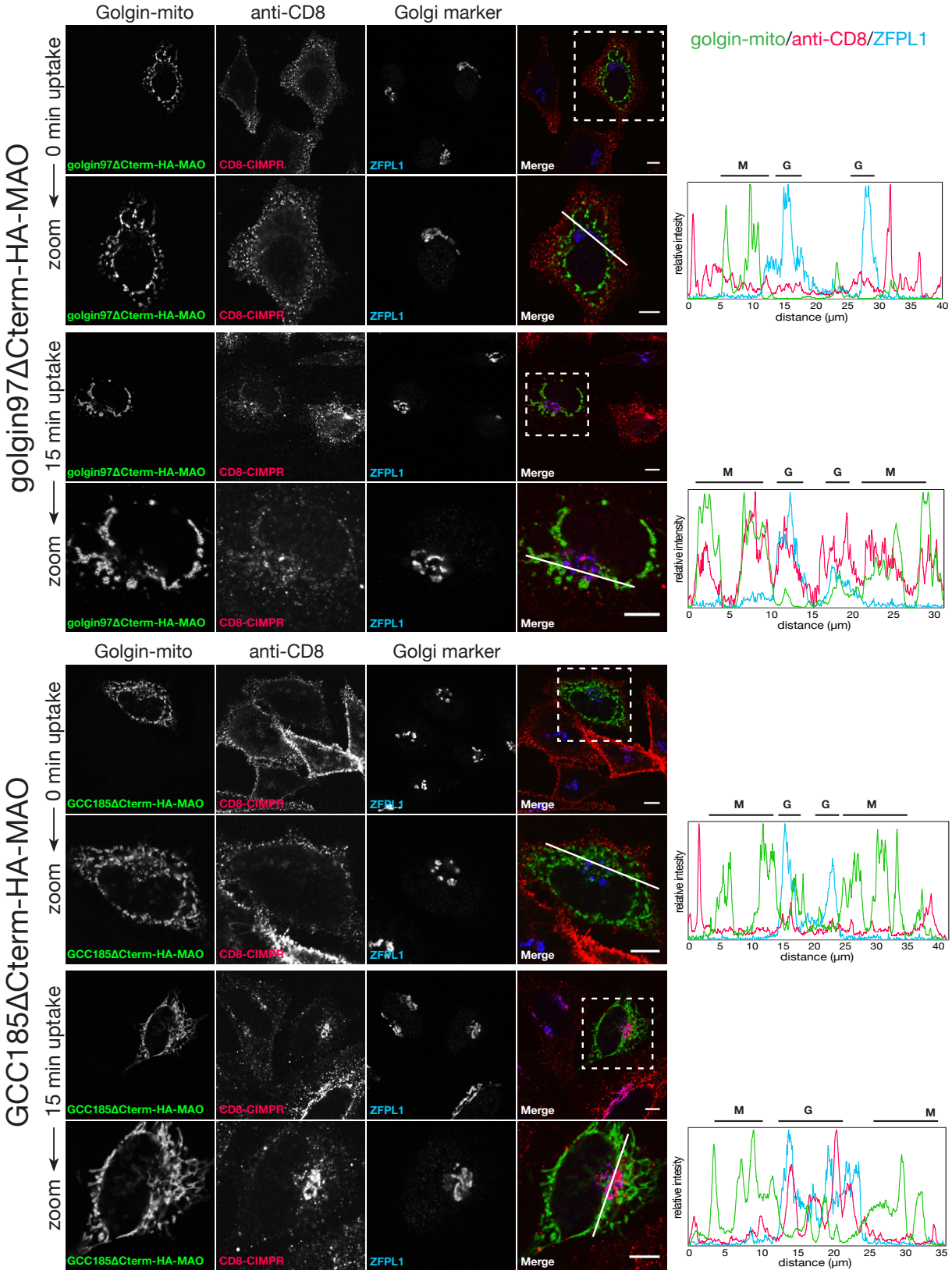

Fig. S6. Internalization of CD8-CIMPR to follow traffic from endosomes to Golgi. Confocal micrographs of CD8-CIMPR-HeLa cells transiently expressing the indicated golgin-mito were labelled with anti-CD8 at 4°C and then shifted to 37°C to induce internalisation of the antibody-chimera complex. Following incubation at 37°C for the indicated times, cells were fixed, permeabilized and costained for the HA epitope on the golgin-mito and ZFPL1 (cis Golgi). Plots of relative intensity against distance show overlap between channels. Anti-CD8 was captured on the mitochondria coated with golgin-97 after only 15 minutes of uptake. Scale bars 10 μm.

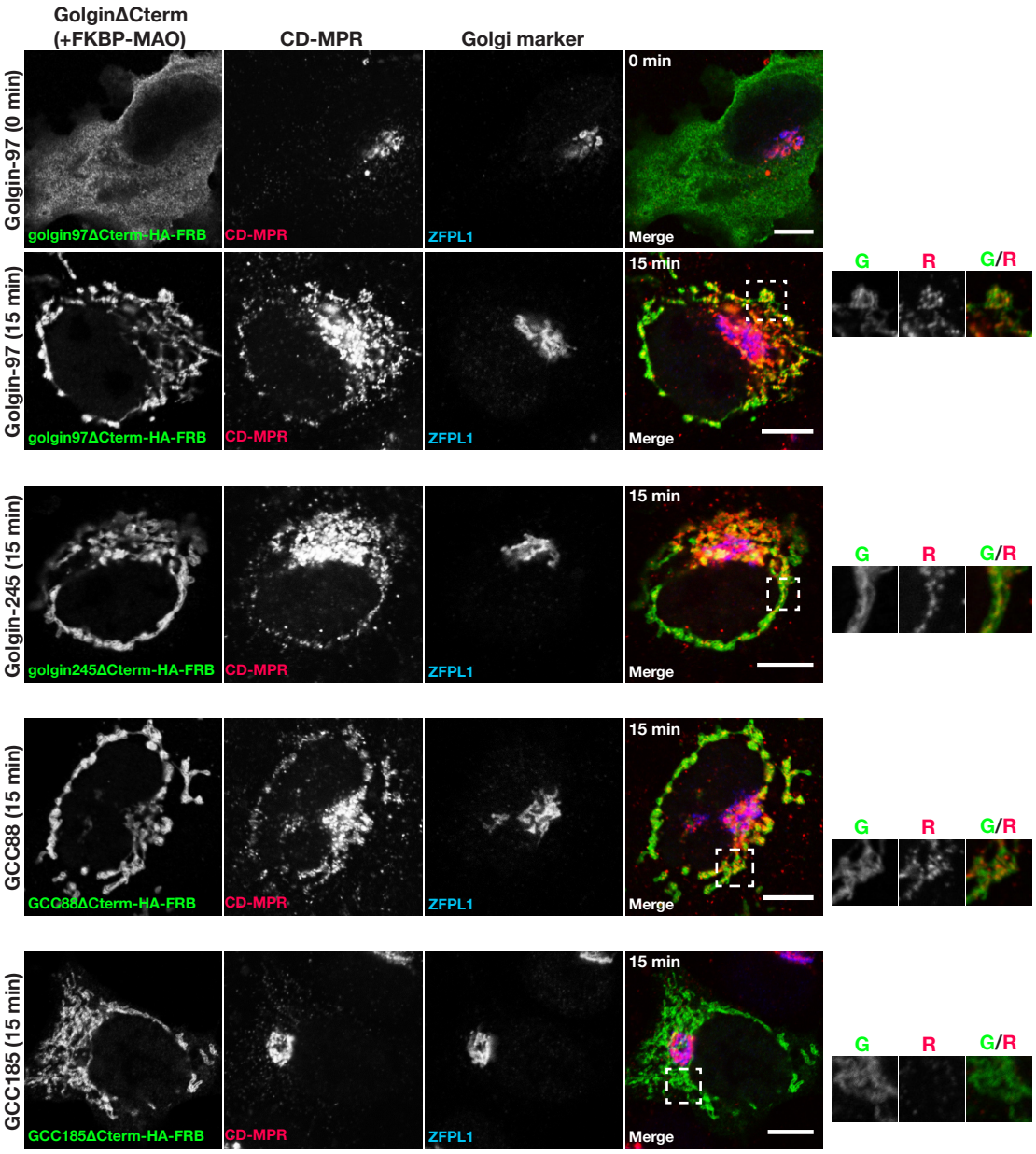

Fig. S7. Golgin-97, golgin-245 and GCC88 rapidly relocate endosome-to-TGN cargo. Confocal micrographs of COS cells coexpressing the indicated reroutable golgin-FRB and FKBP-MAO were treated with 200 nm rapamycin for the indicated times to acutely attach the golgin-FRB to mitochondria. The cells were costained for of the HA epitope on the golgin-FRB, endogenous CDMPR and the Golgi marker ZFPL1 (cis). Representative regions of the cells are shown magnified (Golgi marker omitted; therefore showing green and red channels only). Scale bars 10 µm.

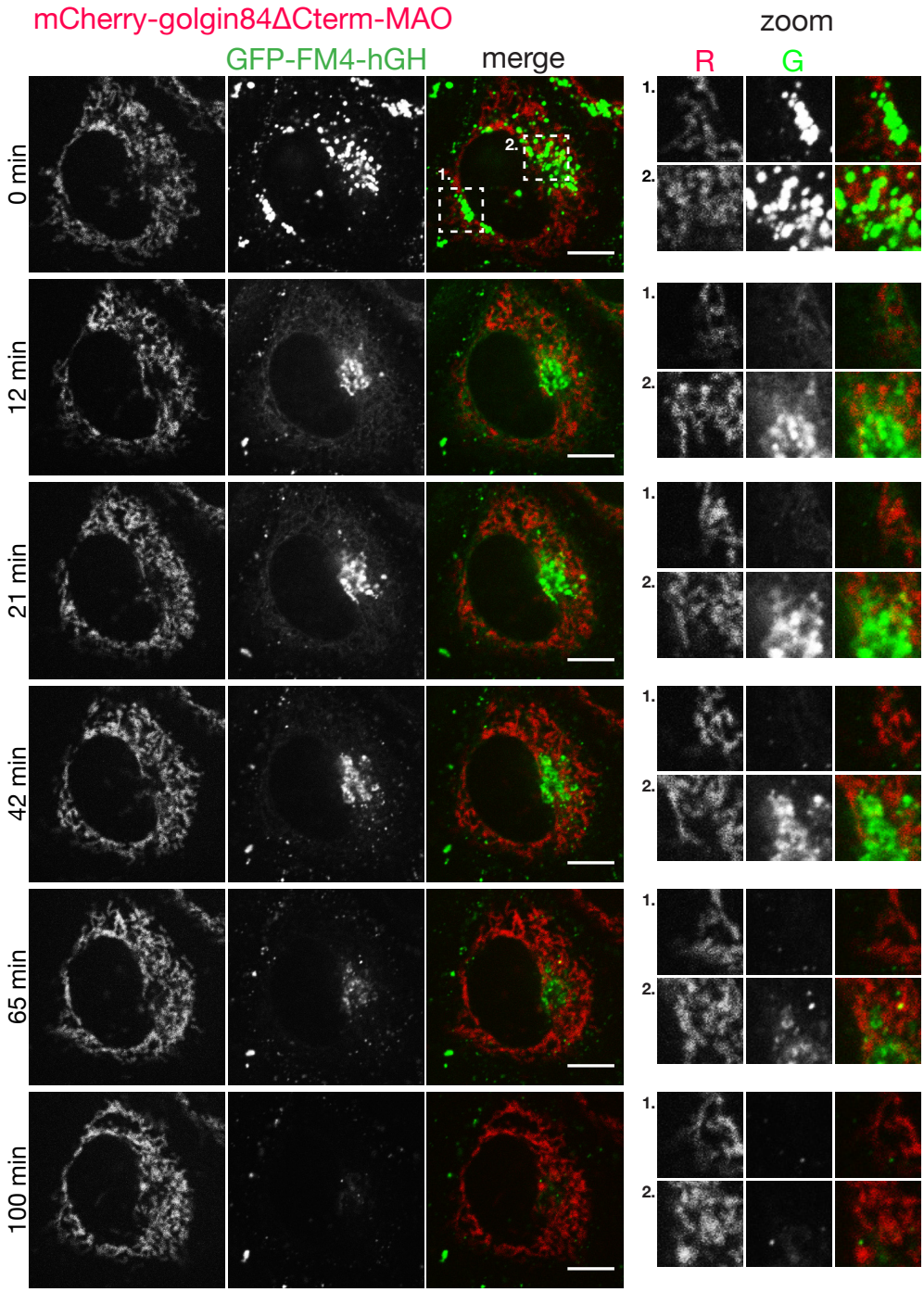

Fig. S8. Golgin-84 does not capture ER-derived cargo on to mitochondria.
Spinning disc micrographs of a C1 cell transiently expressing a mitochondrially-targeted form of mCherry-golgin-84 and subjected to a secretion assay based on release of the cargo GFP-FM4-hGH from the ER using a small molecule ligand. Imaging was initiated directly after ligand addition, with an image acquired every 30 seconds, and representative images shown. Scale bars 5 μm.

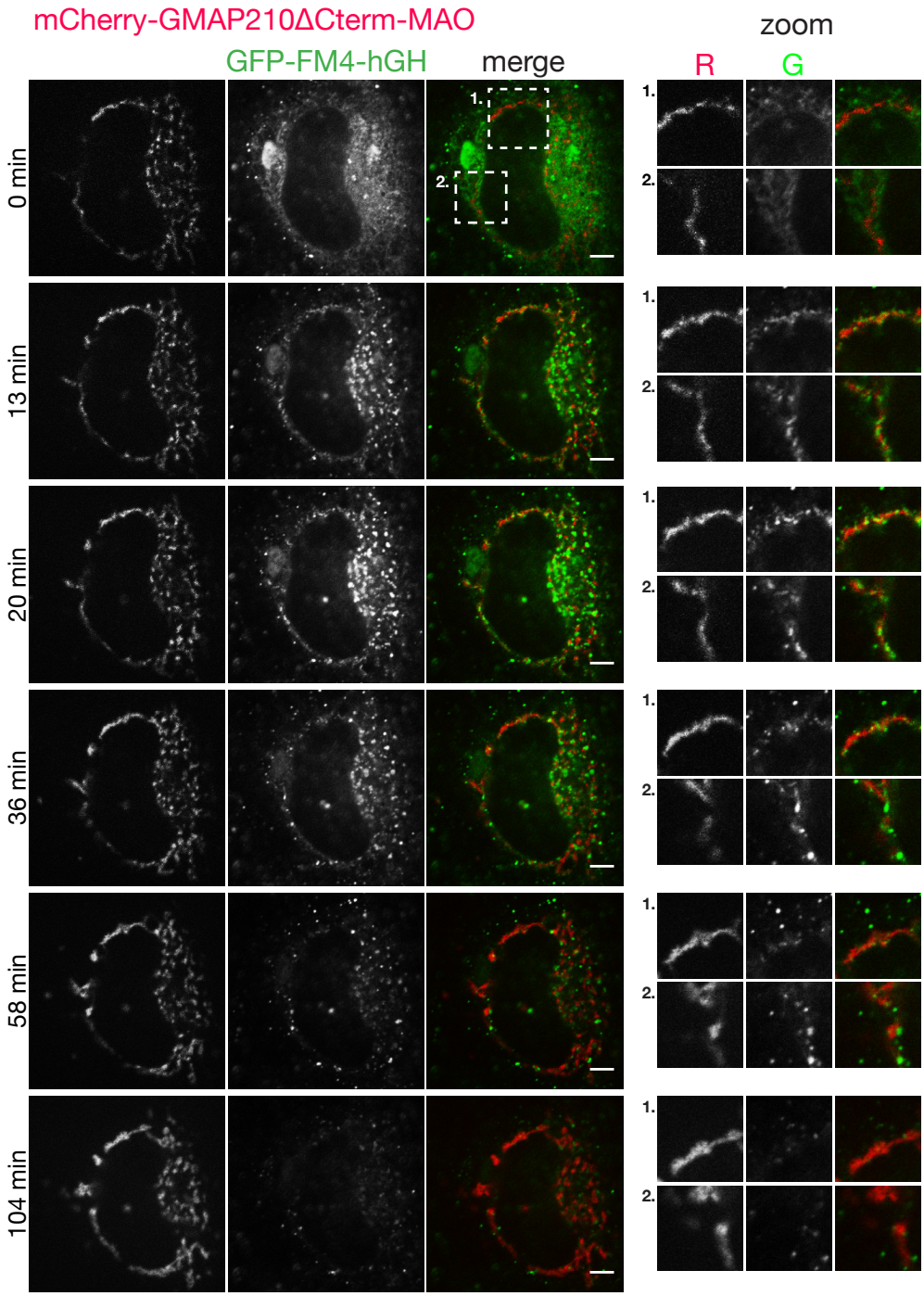

Fig. S9. GMAP210 captures ER-derived cargo on to mitochondria.
Spinning disc micrographs of a C1 cell transiently expressing a mitochondrially-targeted form of mCherry-GMAP210 and subjected to a secretion assay based on release of the cargo GFP-FM4-hGH from the ER using a small molecule ligand. Imaging was initiated directly after ligand addition, with an image acquired every 30 seconds, and representative images shown. Scale bars 5 µm.

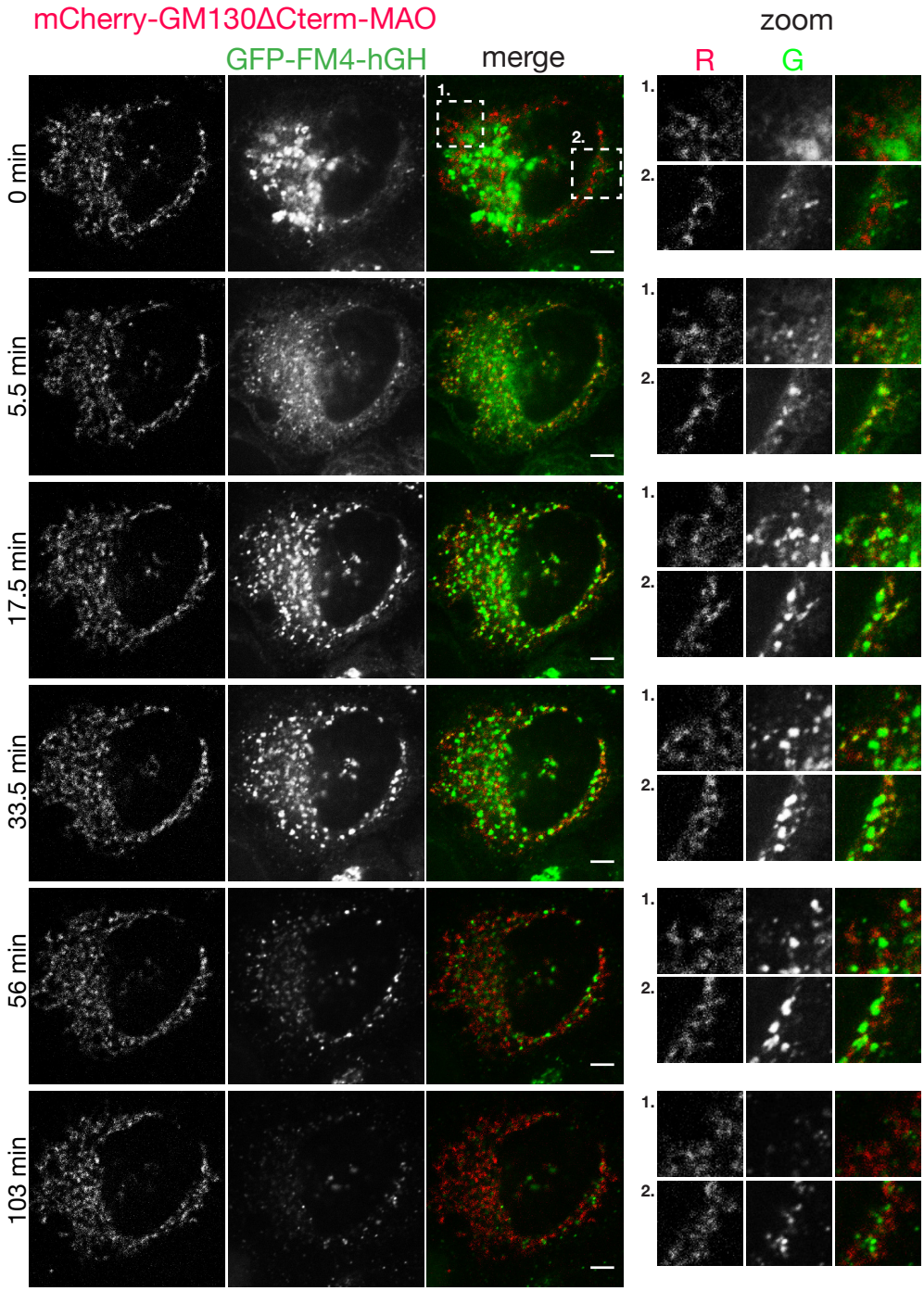

Fig. S10. GM130 captures ER-derived cargo on to mitochondria.
Spinning disc micrographs of a C1 cell transiently expressing a mitochondrially-targeted form of mCherry-GM130 and subjected to a secretion assay based on release of the cargo GFP-FM4-hGH from the ER using a small molecule ligand. Imaging was initiated directly after ligand addition, with an image acquired every 30 seconds, and representative images shown. Scale bars 5 μm.

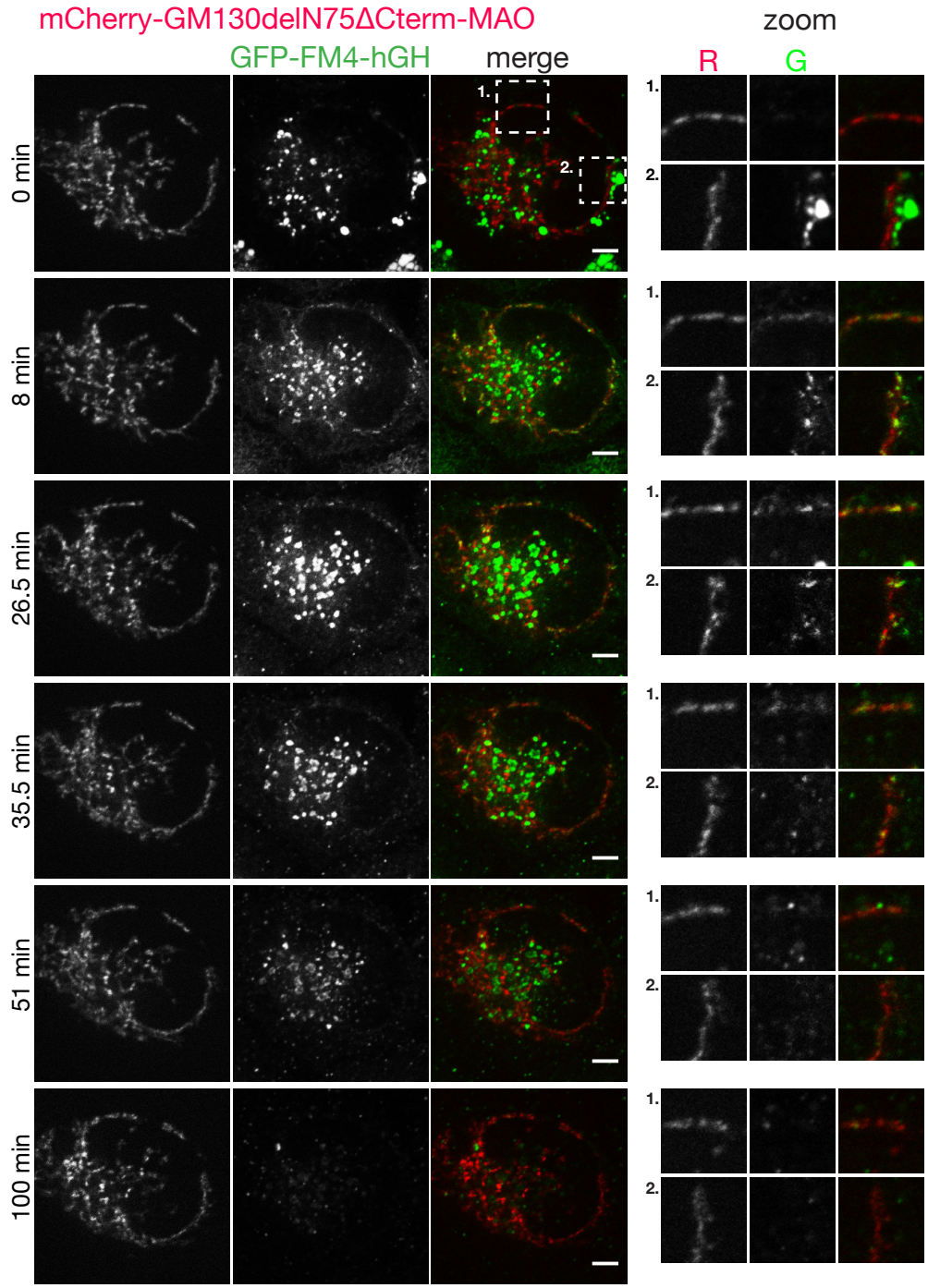

Fig. S11. GM130delN75 captures ER-derived cargo on to mitochondria.
Spinning disc micrographs of a C1 cell transiently expressing a mitochondrially-targeted form of mCherry-GM130delN75 and subjected to a secretion assay based on release of the cargo GFP-FM4-hGH from the ER using a small molecule ligand. Imaging was initiated directly after ligand addition, with an image acquired every 30 seconds, and representative images shown. Scale bars 5 μm.

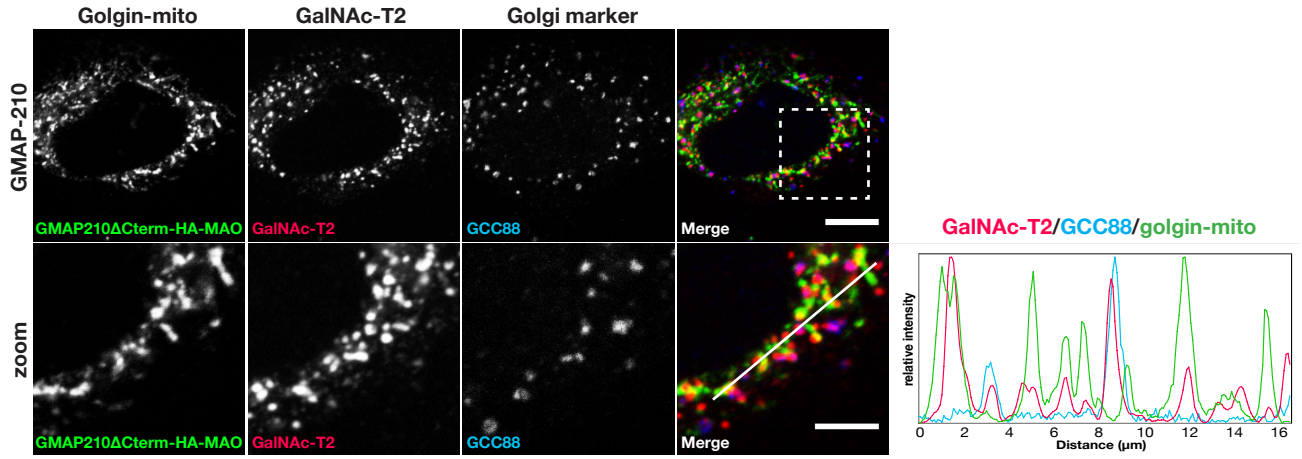

Fig. S12. GMAP210 relocates Golgi resident glycosyltransferases.
Confocal micrographs of COS cells expressing the indicated mitochondrial GMAP-210 and treated with nocodazole for 6 hours prior being fixed and costained for GalNAc-T2 and GCC88 (trans). Plots of relative intensity against distance show occurrence of overlap between channels. Scale bars 10 μm or 5 μm (zoom).

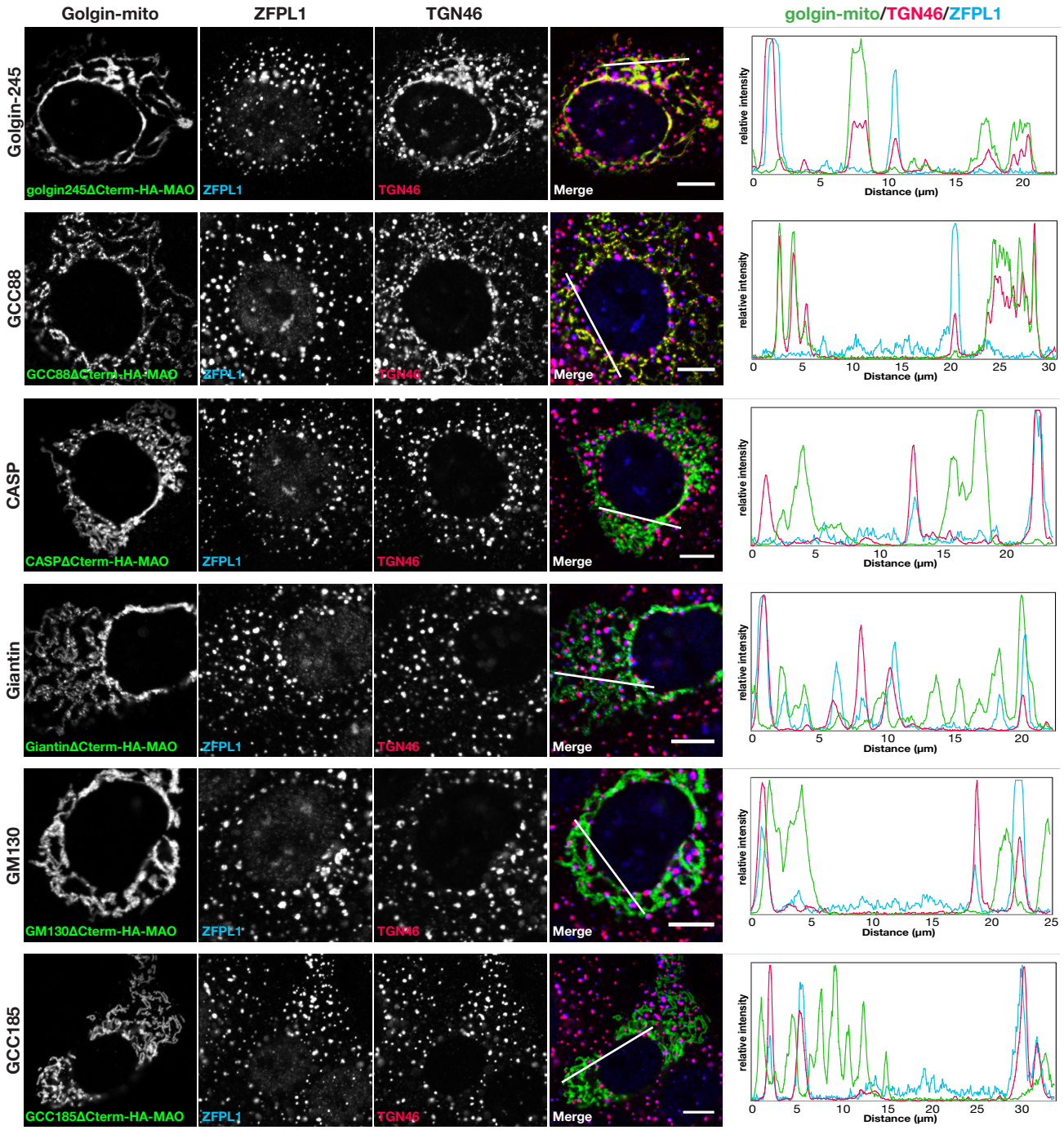

Fig. S13. Most golgins do not relocate Golgi resident proteins.
Confocal micrographs of COS cells expressing the indicated golgin-mito and treated with nocodazole for 6 hours prior to fixation and costaining for two transmembrane Golgi markers, ZFPL1 (cis) and TGN46 (trans). Plots of relative intensity against distance show occurrence of overlap between channels. The membrane protein ZFPL1 has been reported to bind GM130 (28), but is not relocated by the latter. However, the nature and purpose of this interaction is unclear, and it may be that it can occur if the proteins are on the same membrane. Scale bars 10 μm.

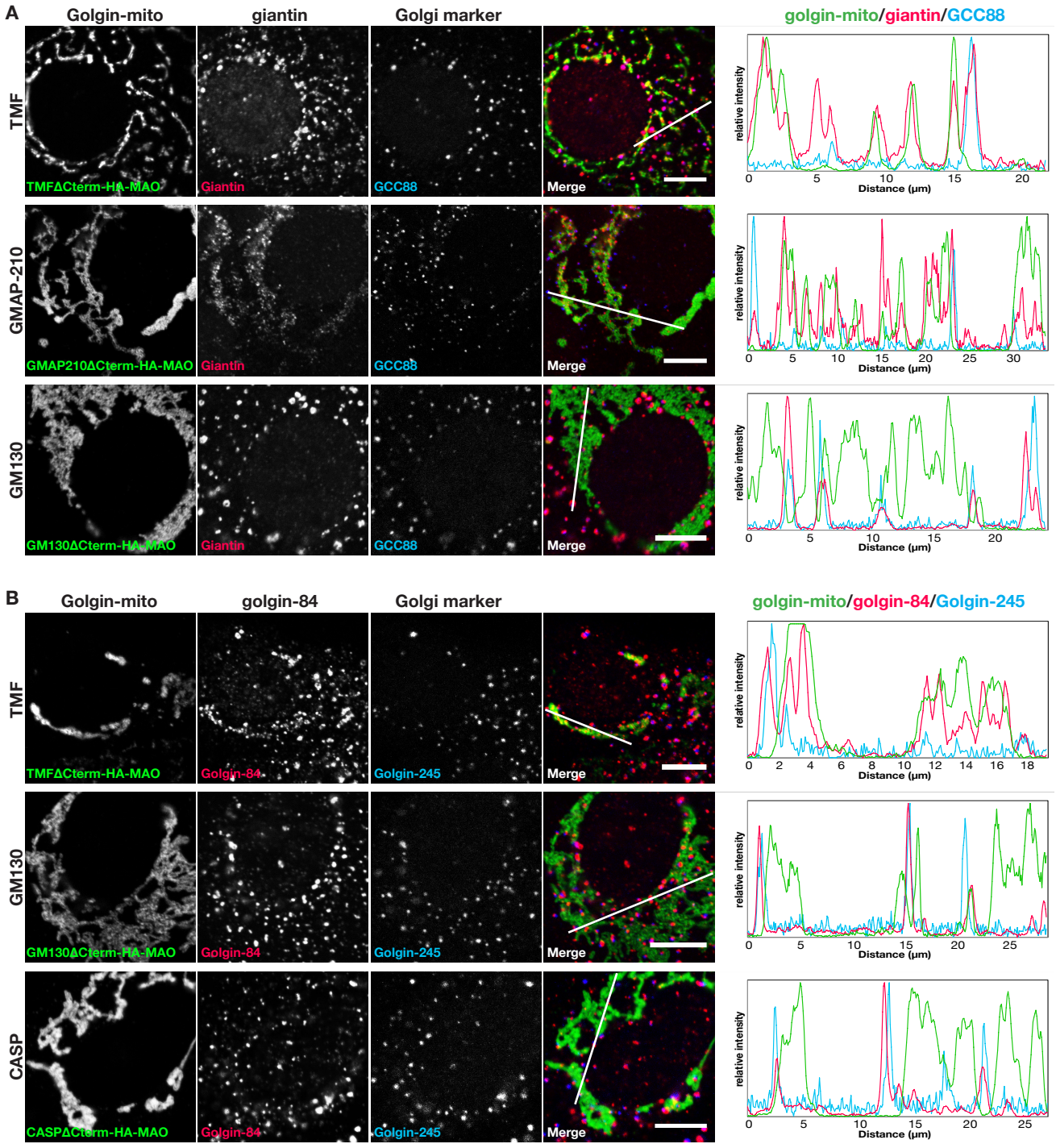

Fig. S14. Relocation of giantin and golgin-84 by a subset of golgins.
(**A**) Confocal micrographs of COS cells expressing the indicated golgin-mito and treated with nocodazole for 6 hours prior to staining for giantin and the Golgi marker GCC88. TMF and GMAP-210, but not GM130, relocated giantin. Plots of relative intensity against distance show occurrence of overlap between channels. Scale bars 10 μm. (**B**) Confocal micrographs of COS cells expressing the indicated golgin-mito and treated with nocodazole for 6 hours prior to staining for the golgin-84, and the Golgi marker, golgin-245. Golgin-84 was relocated by TMF, but not by GM130 or CASP. Intensity plots and scale bars as in (A).

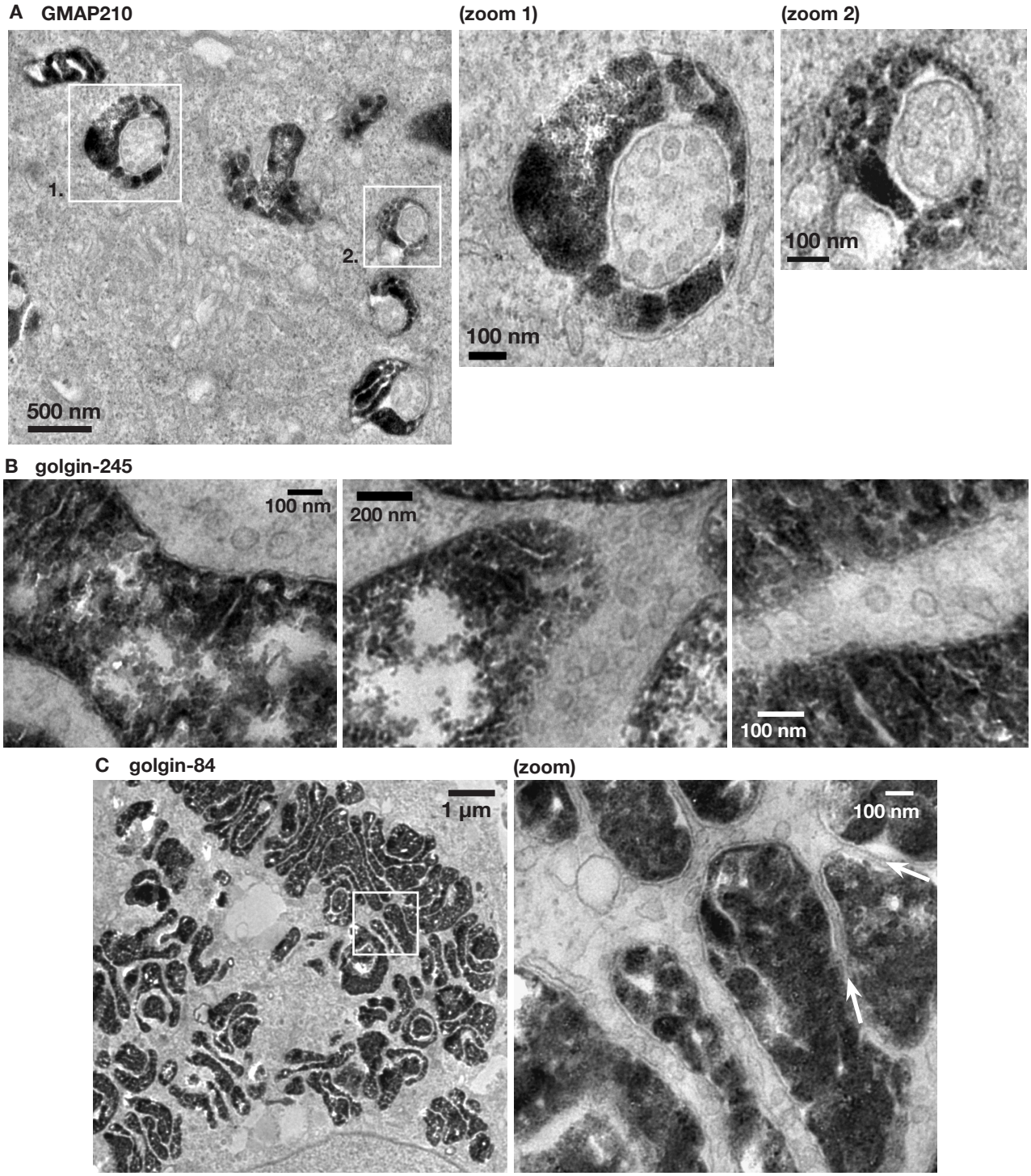

Fig. S15. Ultrastructural analysis of the effect of relocating GMAP-210, golgin-245 or golgin-84 to mitochondria.

(**A**) Electron micrographs of COS cells coexpressing mito matrix-APEX and GMAP-210-mito. The mitochondria frequently curve around the tethered vesicles, but it is currently unclear whether this is a consequence of vesicle capture or reflects a distinct membrane shaping activity of GMAP-210. (**B**,**C**) Electron micrographs of COS cells coexpressing mito matrix-APEX and either golgin-245-mito (B) or golgin-84-mito (C). Vesicles accumulate around mitochondria in both cases, with a preference for the gaps between adjacent mitochondria. With golgin-84 some mitochondria appear closely apposed rather than separated by vesicles (arrows), perhaps due to oligomerization of the protein.

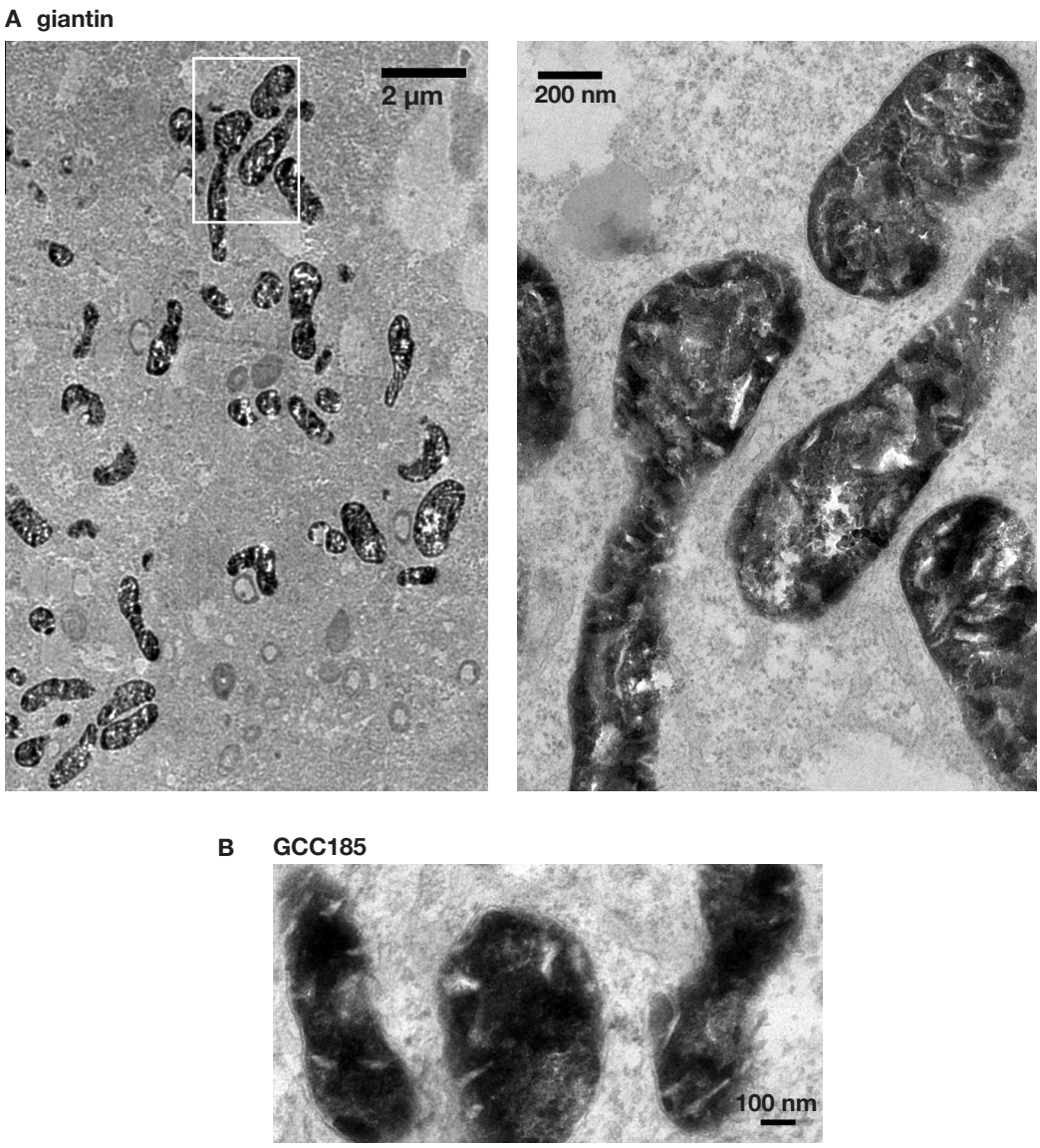

Fig. S16. Ultrastructural analysis of the effect of relocating giantin or GCC185 to mitochondria. (**A**,**B**) Electron micrographs COS cells coexpressing mito matrix-APEX and either giantin-mito (A) or GCC185-mito (B). For neither golgin did we observe an accumulation of associated vesicles.

Movie S1

C1-HeLa cells transiently expressing mitochondria-targeted mCherry-golgin-97ΔCterm-HA-MAO and pre-treated with 0.5 μM nocodazole for three hours to depolymerize microtubules prior to being incubated at 37°C for the indicated times with a mix of nocodazole and D/D solubilizer to induce secretion of the GFP-FM4-hGH reporter. Images were acquired every 2 minutes for 140 minutes. Elapsed time shown in minutes, scale bars 5 μm.

Movie S1

C1-HeLa cells transiently expressing mitochondria-targeted mCherry-GM130ΔCterm-HA-MAO and pre-treated with 0.5 μM nocodazole for three hours to depolymerize microtubules prior to being incubated at 37°C for the indicated times with a mix of nocodazole and D/D solubilizer to induce secretion of the GFP-FM4-hGH reporter. Images were acquired every 2 minutes for 140 minutes. Elapsed time shown in minutes, scale bars 5 μm.