Supplemental Materials

Molecular Biology of the Cell

Benyair et al.

Mammalian ER mannosidase I resides in quality control vesicles where it encounters its glycoprotein substrates

Ron Benyair, Navit Ogen-Shtern, Niv Mazkereth, Ben Shai, Marcelo Ehrlich and Gerardo Z. Lederkremer*

Supplemental information:

Supplementary video legends:

Movie S1. HeLa cell expressing GalT-YFP and ERManI-cherry showing QCV movement.

Movie S2. HEK 293 cell expressing GalT-YFP and ERManI-cherry showing QCV movement.

Movie S3. NIH 3T3 cell expressing ERManI-cherry, showing QCV movement.

Movie S4. NIH 3T3 cell expressing GalT-YFP and ERManI-cherry, showing QCV movement.

Movie S5. NIH 3T3 cell expressing GalT-YFP and ERManI-cherry treated with Nocodazole, showing QCV clumping and lack of mobility.

Movie S6. NIH 3T3 cell expressing GalT-YFP and ERManI-cherry treated with BFA, showing no effect on QCV mobility.

Movie S7. NIH 3T3 cell expressing YFP-GBF1[E794K] and ERManI-cherry, showing no effect of the dominant negative GBF1 on QCV mobility.

Movie S8. NIH 3T3 cell expressing GalT-YFP and ERManI-cherry treated with H89, showing arrest in QCV mobility despite retaining a vesicular pattern.

Movie S9. NIH 3T3 cell expressing Sar1[T39N]-IRES-GFP and ERManI-cherry, showing an inhibitory effect on mobility of a sub-population of QCVs.

Movie S10. NIH 3T3 cell expressing H2a-GFP and ERManI-cherry treated with Lac, showing convergence of ERManI-cherry to the juxtanuclear ERQC along with H2a-GFP over time (h:min:sec).

Benyair et al Fig. S1

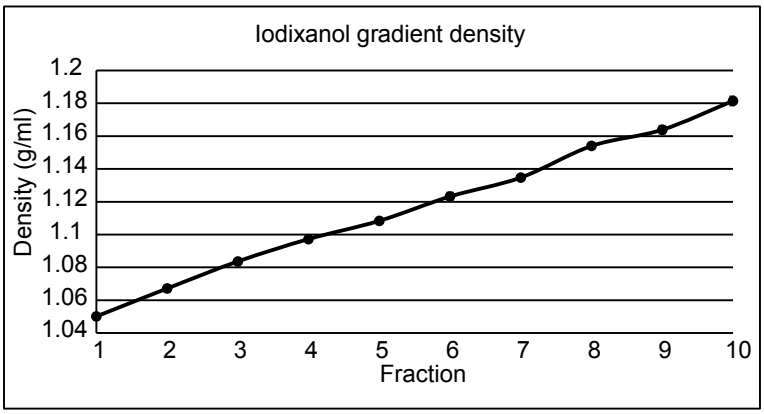

Fig. S1. Density of iodixanol gradients. Densities of iodixanol gradient fractions as extrapolated from the refraction index of each fraction. The graph is the average of the gradients shown in the main figures. Standard errors of densities between experiments were between 0.000823 and 0.002375 g/ml.

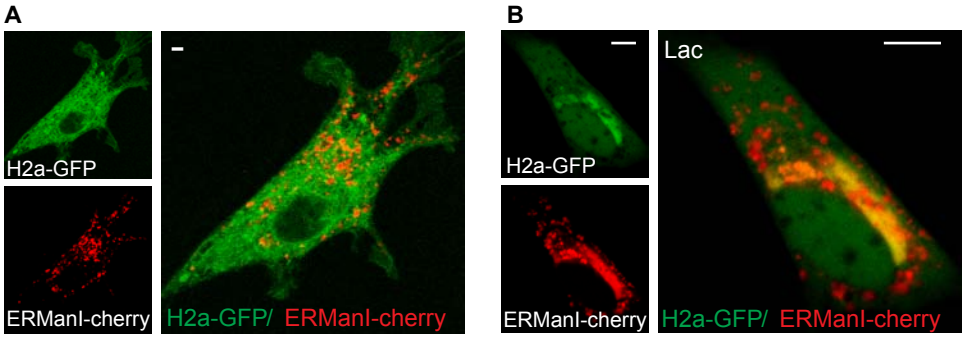

Fig. S2. Localization of ERManI and a substrate glycoprotein upon proteasomal inhibition. Live cell imaging of NIH 3T3 cells expressing ERManI-cherry and H2a-GFP. When left untreated (A), no colocalization is apparent but under proteasomal inhibition by 17μM lac for 3 hours (B), both ERManI and the ERAD substrate colocalize at the ERQC. Bars= 10μm.

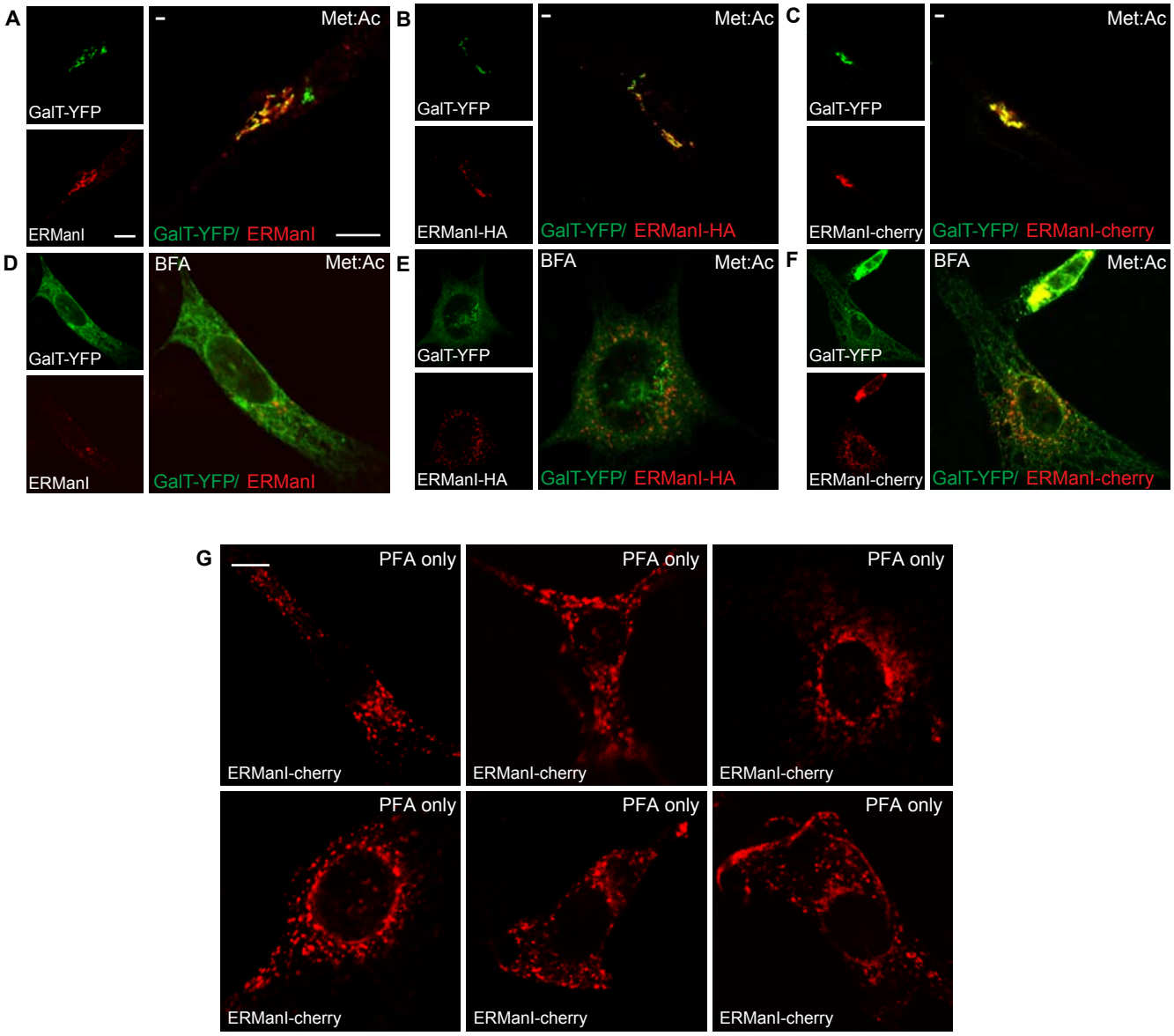

Fig. S3. Alteration of ERManI localization following cell fixation and permeabilization. A-C) Endogenous ERManI (A), ERManI-HA (B) and ERManI-cherry (C) colocalize with GalT-YFP in NIH 3T3 cells fixed and permeabilized by the methanol:acetone method (Materials and methods). Staining as in Fig. 4. Bars=10μm. **D-F)** GalT-YFP separates from endogenous ERManI (D), ERManI-HA (E) or ERManI-cherry (F) after BFA treatment, similarly to what was observed in cells fixed with 3% PFA and permeabilized with 0.5% triton (Fig. 4). **G)** Several NIH 3T3 cells fixed with PFA and left unpermeabilized, showing in these conditions a vesicular pattern of ERManI-cherry like in live cells.

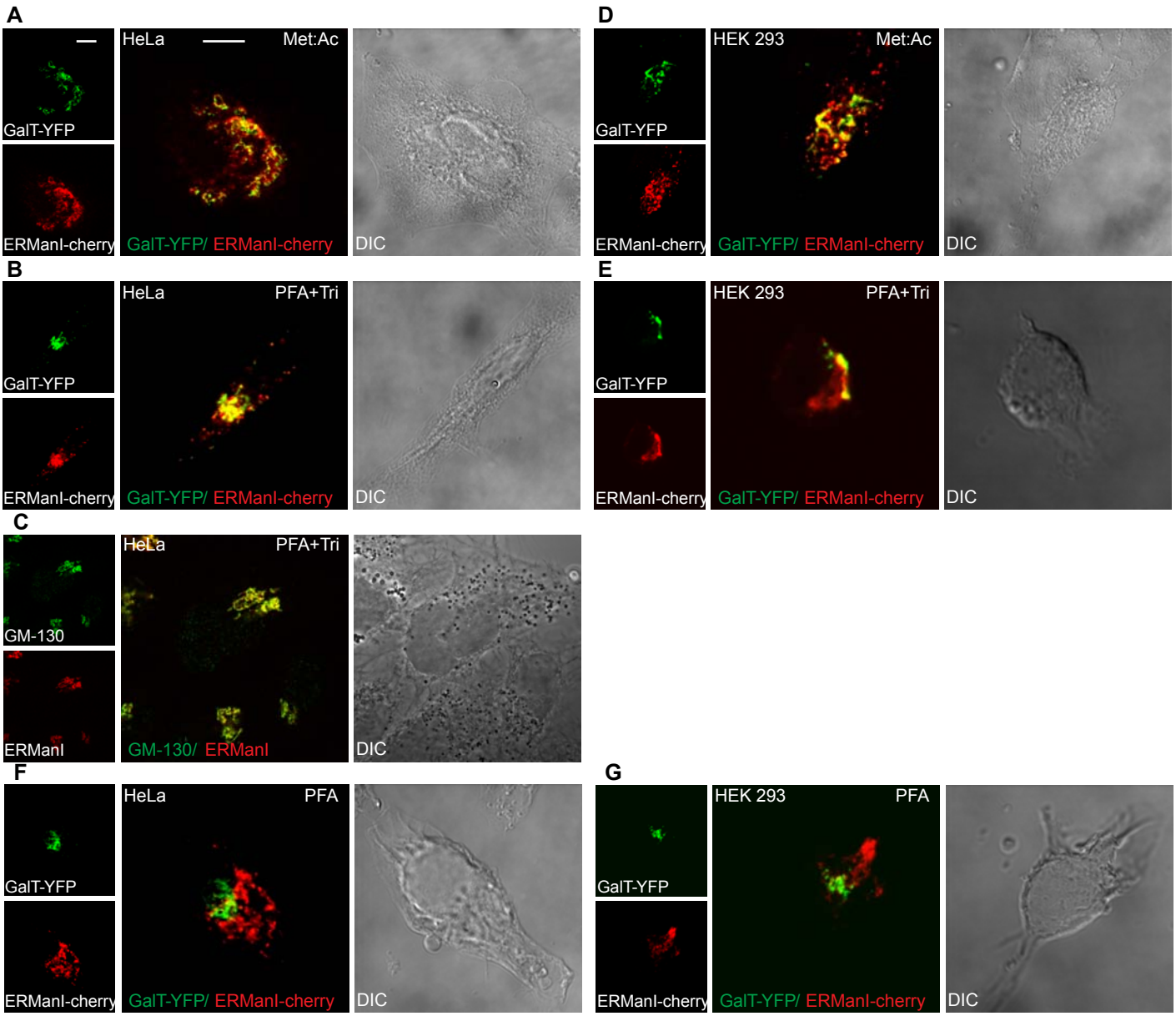

Fig. S4. Alteration of ERManI localization in HeLa and HEK 293 cells following cell fixation and permeabilization. A-C) In HeLa cells, high levels of colocalization are observed between ERManI-cherry and GalT-YFP when fixed and permeabilized with methanol:acetone 1:1 (A) or with 3% PFA + 0.5% triton (B). Similarly between endogenous ERManI and the endogenous Golgi marker GM-130 (C). Bars=10μm. **D-E)** A lower degree of colocalization of ERManI-cherry with H2a-GFP is apparent in HEK 293 cells after fixation and permeabilization. **F-G)** When HeLa or HEK 293 cells were fixed with PFA and left unpermeabilized there was little or no colocalization.

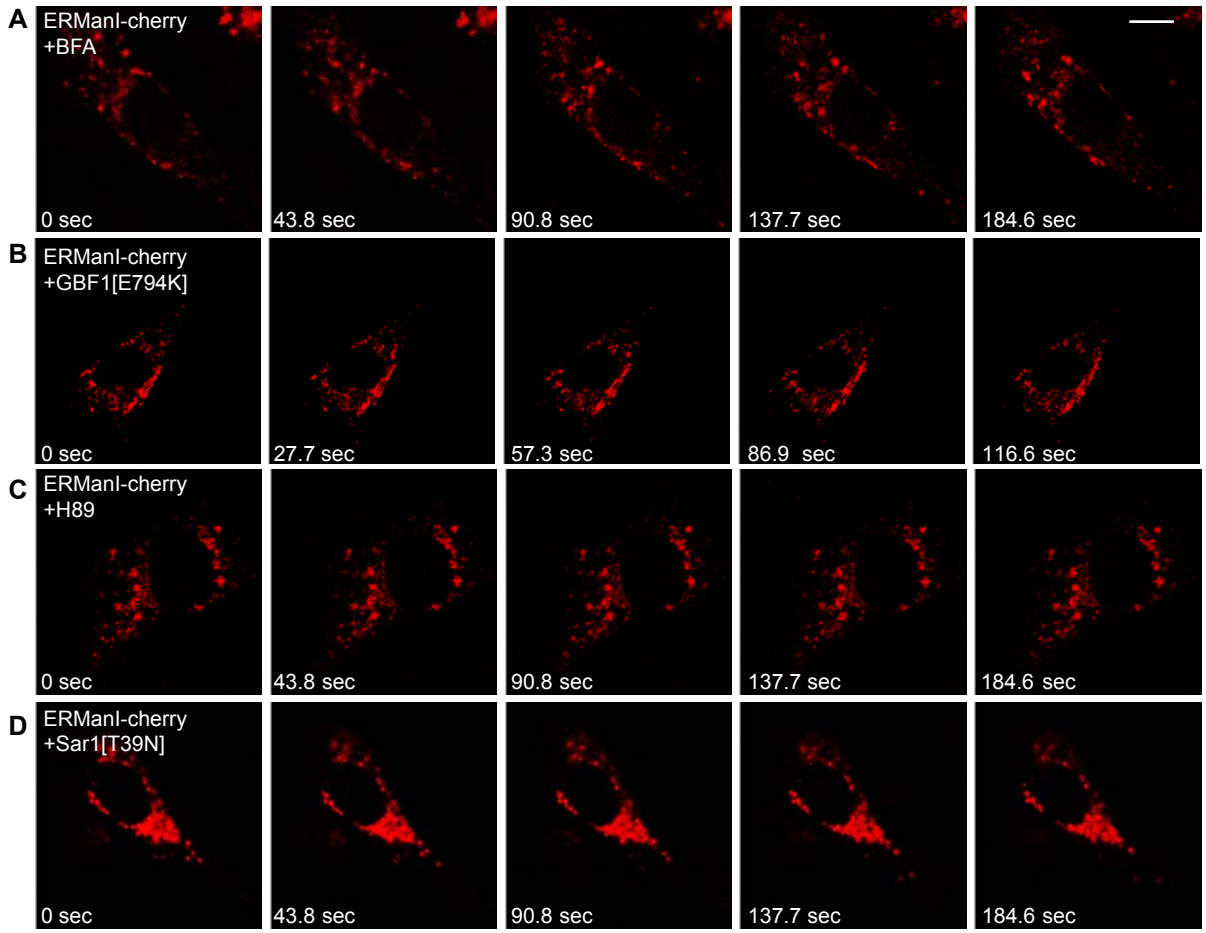

Fig. S5. QCV dynamics. A) Live cell microscopy time-lapse images of NIH 3T3 cells expressing ERManI-cherry and treated with 5μg/ml BFA for 1 hour showed no effect on ERManI vesicle mobility. Bar=10μm. **B)** Similar to (A) but with cells expressing dominant negative YFP-GBF1[E794K]. **C-D)** Arrest of vesicle mobility is evident in cells treated with 50μM of H89 for 1 hour (C) and to a lesser extent in cells expressing dominant negative Sar1[T39N] (D).