Supplemental Materials

Molecular Biology of the Cell

Hellerschmied et al.

Supplemental Information

Protein Folding State-dependent Sorting at the Golgi Apparatus

Doris Hellerschmied[1*†], Yevgeniy V. Serebrenik[1†], Lin Shao[4], George M. Burslem[1], and Craig M. Crews*[1, 2, 3]

1) Department of Molecular, Cellular and Developmental Biology, 2) Department of Chemistry, and 3) Department of Pharmacology, Yale University, New Haven, CT 06511

4) Department ofNeuroscience, Yale School of Medicine, New Haven, CT 06520

[†]These authors contributed equally to the work.

Co-corresponding author: doris.hellerschmied-jelinek@yale.edu

Correspondence to: craig.crews@yale.edu

Content: Supplemental Figures 1-6, Supplemental Movies 1-4

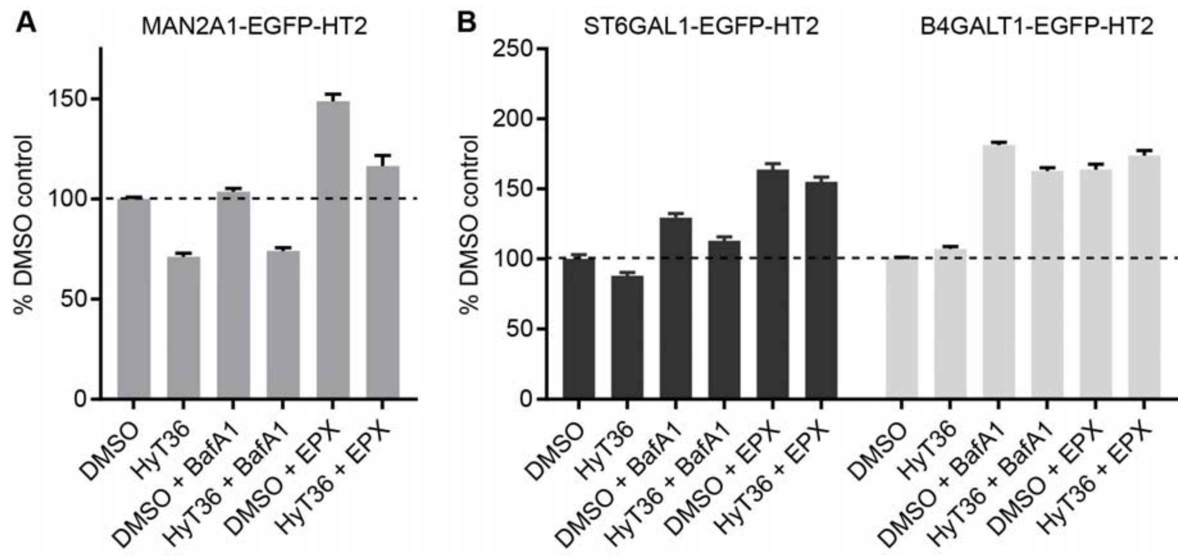
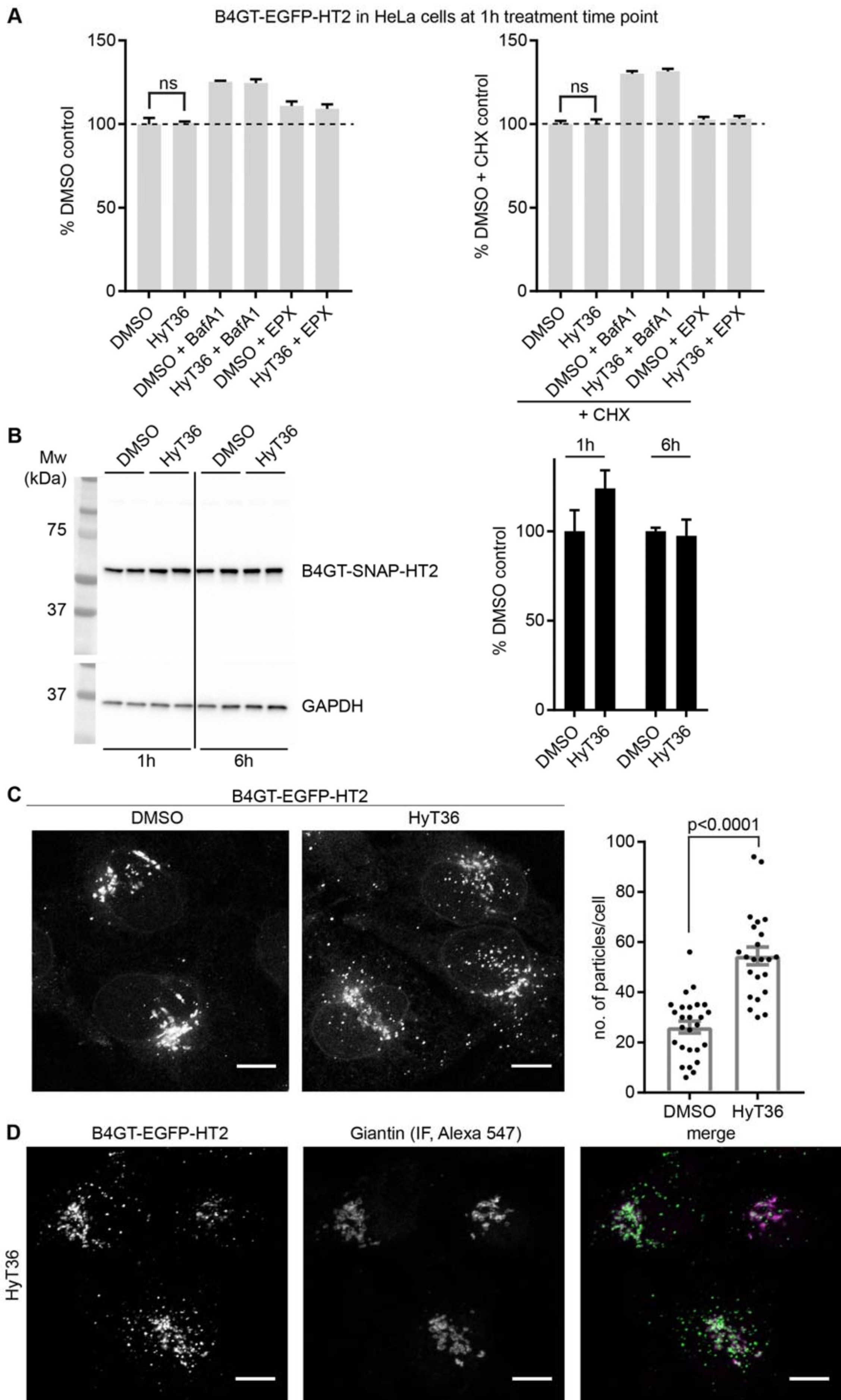


Figure S1. Flow cytometry analysis of Golgi-targeted EGFP-HT2 constructs in HEK293 cells.(A) MAN2A1-EGFP-HT2 expressing HEK293 cells or (B) ST6GAL1-EGFP-HT2 and B4GALT1-EGFP-HT2 were treated with the indicated compounds for 6h. All data are normalized to DMSO control. (n = 2, data represent mean ± s.e.m., EPX - epoxomicin, BafA1 – bafilomycin A)



A
B4GT-EGFP-HT2 in HeLa cells at 1h treatment time point
% DMSO control
% DMSO + CHX control
ns
ns
DMSO
HyT36
DMSO + BafA1
HyT36 + BafA1
DMSO + EPX
HyT36 + EPX
+ CHX
B
Mw (kDa)
75
37
37
B4GT-SNAP-HT2
GAPDH
1h
6h
C
B4GT-EGFP-HT2
DMSO
HyT36
p<0.0001
no. of particles/cell
D
B4GT-EGFP-HT2
Giantin (IF, Alexa 547)
merge
HyT36

Figure S2. Formation of QC carriers containing unfolded Golgi protein. (A) Flow cytometry analysis quantifying the levels of B4GT-EGFP-HT2 in HeLa cells upon the indicated treatments for 1h in the absence (left) or presence (right) of cycloheximide (CHX). CHXwas added 1h prior to the indicated treatments. (n = 3, data represent mean ± s.e.m., EPX - epoxomicin, BafA1 – bafilomycin A, results of t-test are shown) (B) Left panel: anti-HA western blot showing the levels of B4GT-SNAP-HT2 and the loading control GAPDH in HeLa cells treated with DMSO (control) or HyT36 for 1h or 6h. Right panel: Quantification of B4GT-SNAP-HT2 levels normalized to GAPDH and DMSO. (C) Left panel: Representative confocal microscopy images of HeLa cells expressing B4GT-EGFP-HT2 treated with DMSO (control) or HyT36 for 4h at 20°C followed by 1h at 37°C. Right panel: Particles per cell were quantified in FIJI (n=26 for DMSO, n=23 for HyT36). (D) B4GT-EGFP-HT2-expressing HeLa cells were treated with HyT36 for 4h at 20°C followed by 1h at 37°C followed by immunofluorescence staining of Giantin using a secondary antibody conjugated to Alexa547. All images are maximum projections of z-stacks. Scale bars correspond to 10 µm. Bar graphs represent mean ± s.e.m., p-values (t-test) are shown.

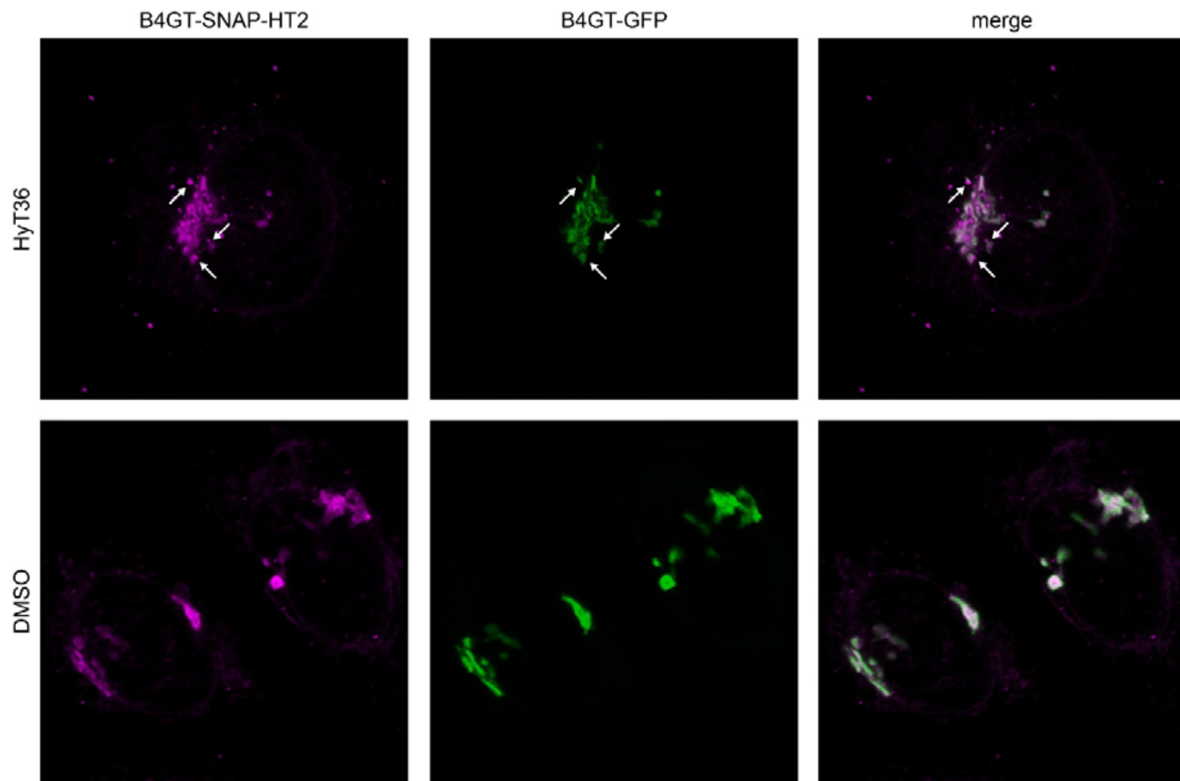


Figure S3. HyT36 induces sorting of HT2-fusion proteins at the Golgi. HeLa cells expressing B4GT-SNAP-HT2 and B4GT-GFP were treated with DMSO (control) or HyT36 for 45 min at 37°C.Clear overlap of the magenta and green signal is observed under control conditions. Arrows highlight separation of B4GT-GFP and B4GT-SNAP-HT2 in HyT36 treated cells. All images are 3D projections of z-stacks.

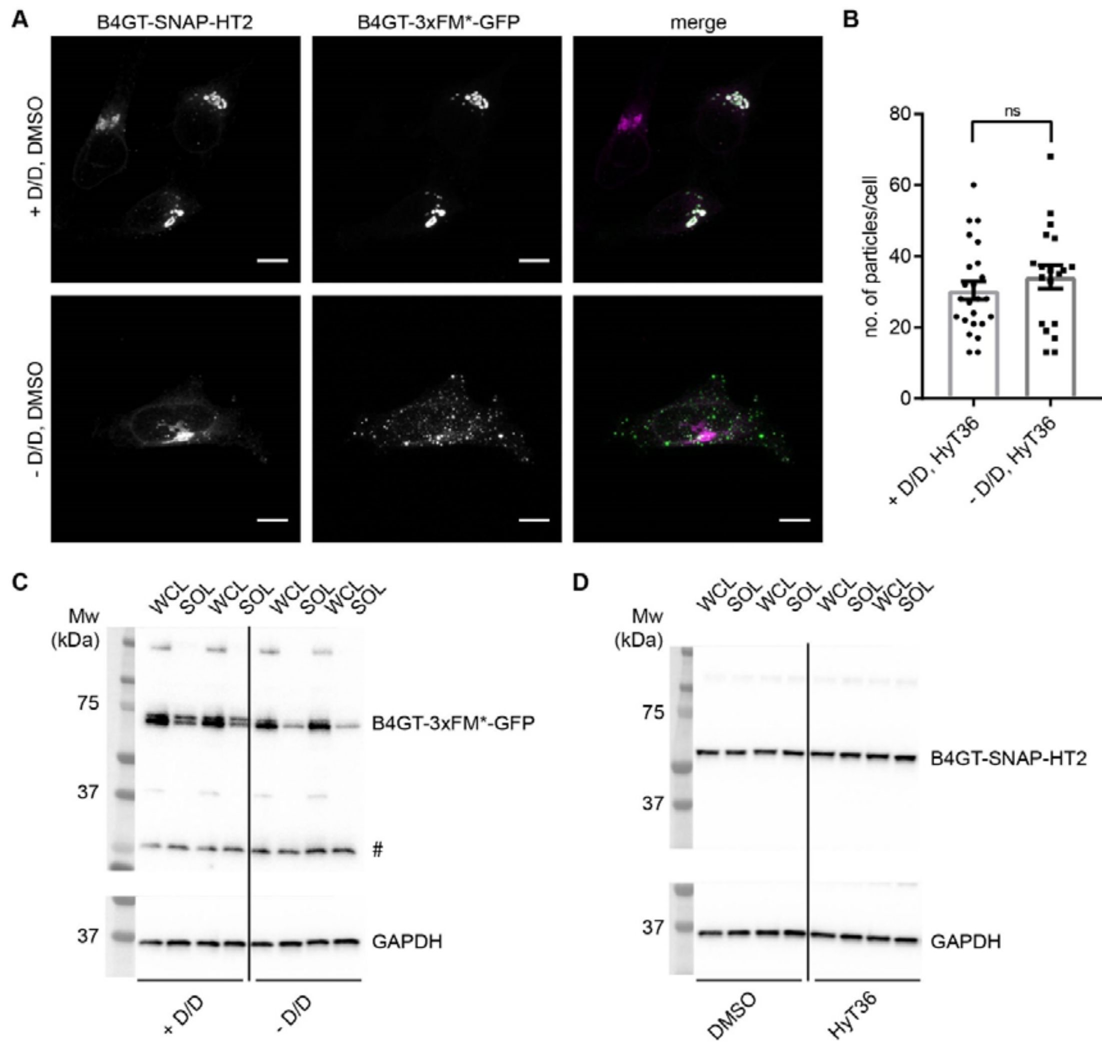


Figure S4. Protein aggregation induces Golgi export. (A) B4GT-SNAP-HT2 expressing HeLa cells were transfected with B4GT-3xFM*-GFP. Cells were treated with DMSO control in the presence or absence of D/D solubilizer for 4h at 20°C followed by 1h 37°C. (B) Quantification of B4GT-SNAP-HT2 particles per cell. The total number of particles does not show a significant difference (t-test) between the presence or absence of the D/D solubilizer. Percent of GFP-positive particles are shown in Fig. 4B. Bar graphs represent mean ± s.e.m. (C) Aggregation analysis of B4GT-3xFM*-GFP. Anti-GFP Western blot showing whole cell lysate (WCL) and soluble fraction after centrifugation (SOL). (#) highlights a degradation product, likely corresponding to free GFP based on its size. GAPDH, a soluble cytosolic protein, is used as loading control. (D) Same analysis as in (C) performed for B4GT-SNAP-HT2 (visualized by anti-HA western blot).

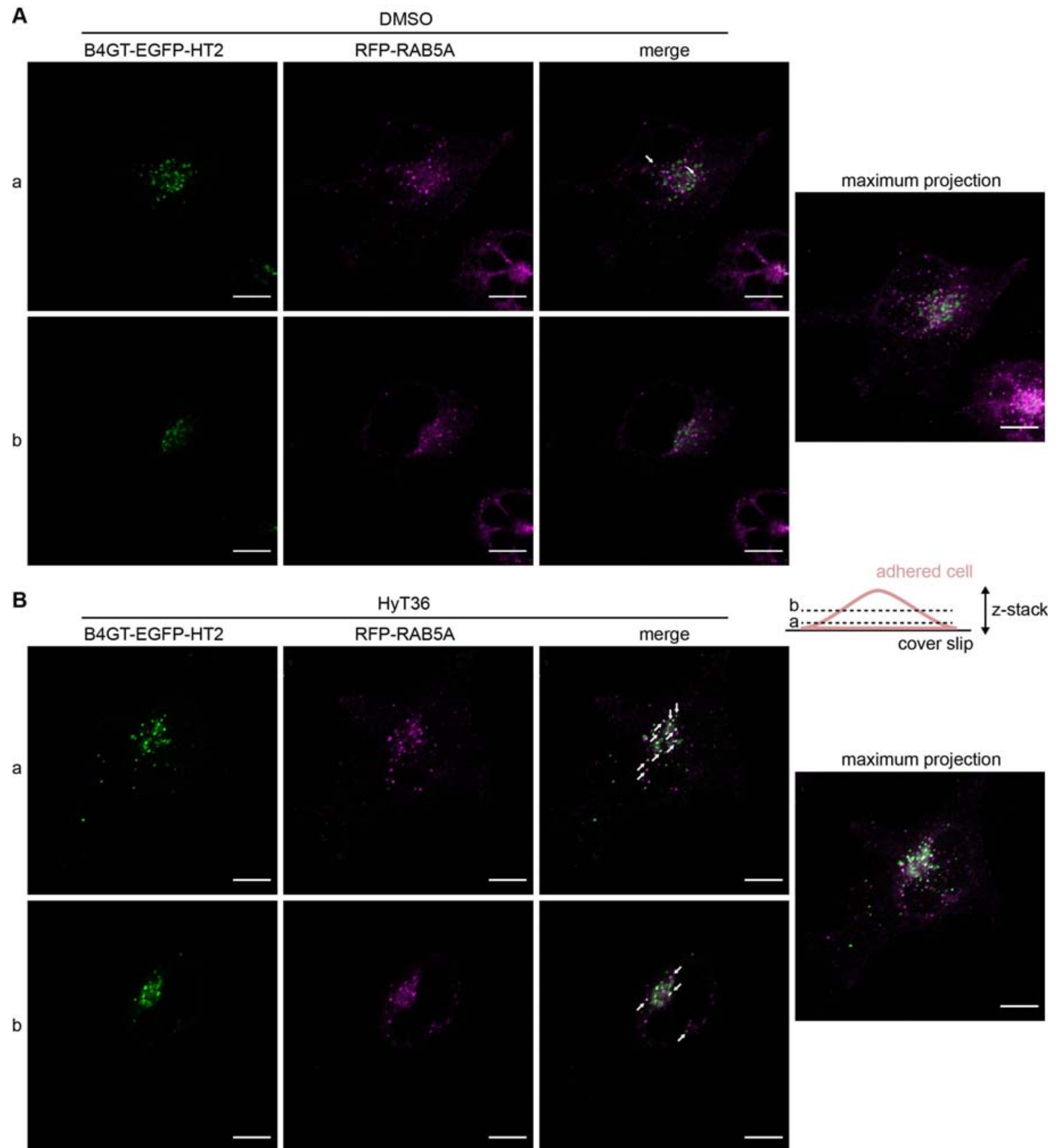


Figure S5. RFP-RAB5A labels B4GT-EGFP-HT2 containing carriers. B4GT-EGFP-HT2-expressing HeLa cells were transfected with RFP-RAB5A. Single slices and maximum projections of z-stacks for cells treated for 4h at 20°C and 1h at 37°C with DMSO (A) orHyT36 (B)are shown. Cartoon illustrates the individual slices of the z-stack (a and b).Carriers labelled with RFP-RAB5A are highlighted with white arrows.

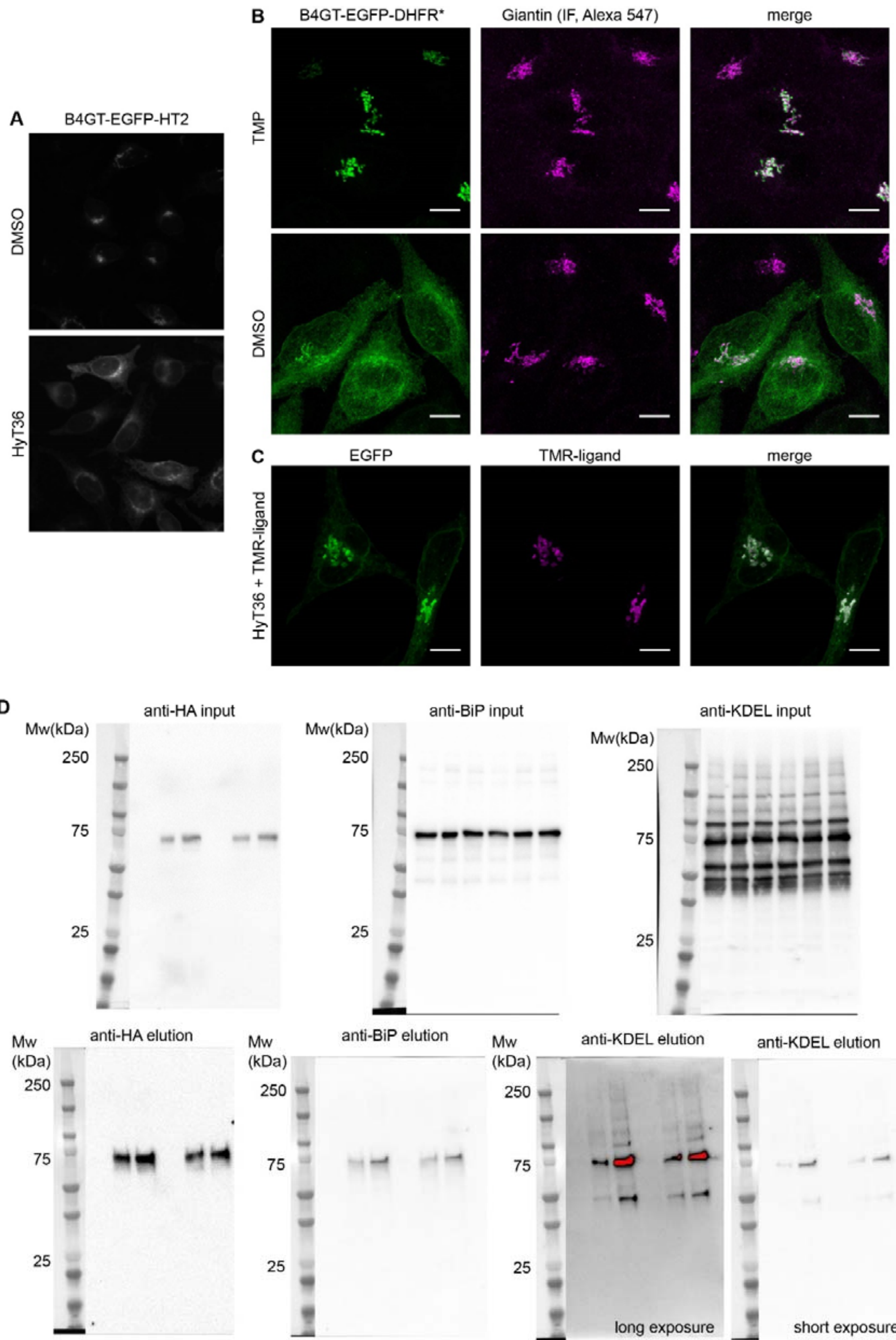


Figure S6. Unfolded Golgi proteins localize to the endoplasmic reticulum. (A) Example images showing B4GT-EGFP-HT2-expressing HeLa cells treated with HyT36 or DMSO for

4h at 37°C, which were used for the quantification presented in Fig. 6C. (B) B4GT-EGFP-DHFR*-expressing HeLa cells were incubated with the stabilizer TMP or TMP washed out and treated with DMSO for 3.5h at 37°C. The Golgi was visualized by immunofluorescence staining of Giantin using a secondary antibody conjugated to Alexa 547. (C) B4GT-EGFP-HT2-expressing HeLa cells were treated with HyT36 and a HaloTag TMR ligand for 5h at 37°C. Images are maximum projections of z-stacks. Scale bars correspond to 10 μm. (D) Uncropped western blots for Fig. 6D.