Supplemental Material Bagley et al 5_7_12

Supplemental Figure 1 Reductions in Mon1a do not affect endocytosis or lysosome morphology.

Cells silenced with nonspecific or Mon1a specific oligonucleotides were given Texas Red dextran overnight followed by a two h chase, which has been previously shown to accumulate in lysosomes (11). Cells were then incubated with Alexa488-Tf (green) for 30 min, washed and imaged using confocal and epifluorescence microscopy. Scale bar = 10 μm. Vesicle size was measured using Image J software where fluorescent vesicle area was measured as described previously (15). The graph represents the average Alexa488-Tf-positive vesicle size (>400 vesicles) or the average Alexa594-dextran-positive vesicle size (>400 vesicles) of 50-100 cells from three separate experiments.

Supplemental Figure 2 Overexpression of mMon1a or mMon1b does not suppress the Δ*mon1* or Δ*ccz1* vacuole fragmentation, high zinc sensitivity nor CPY secretion.

A. Wild type (BY4743), Δ*mon1* or Δ*ccz1* cells transformed with pYES2.0, pYES2.0 FLAG-Mon1a or pYES2.0 FLAG-Mon1b were grown to log-phase in galactose containing CM-ura. Ten ODs of cells were pelleted, incubated with 5 μM FM4-64 for 10 min, washed and chased in growth medium for an additional 45 min. Cells were mounted onto glass slides and imaged using an epifluorescence microscope with a 100X 1.4NA oil immersion objective. Images were acquired using Pictureframer software (Olympus Inc., Melville, NY). All experiments were performed a minimum of three times and representative images are shown. B. Cells grown as in A were serial diluted, spotted onto CM-ura galactose plates supplemented with 5 mM $ZnCl_2$ and grown for two days at 30ºC. Cells from B were lysed and trichloroacetic acid precipitated as described in Materials and Methods, resolved by SDS-PAGE and FLAG-tagged YMon1, mMon1a and mMon1b detected by Western blot analysis. C. Wild type and Δ*mon1* cells as in A were spotted onto CM-ura galactose plates, grown for one day, nitrocellulose overlayed and cells grown for an additional 24 h. Nitrocellulose membranes were washed and Western blot analysis performed using mouse-anti-CPY as the primary antibody followed by peroxidase-conjugated goat-anti-mouse IgG. N=5.

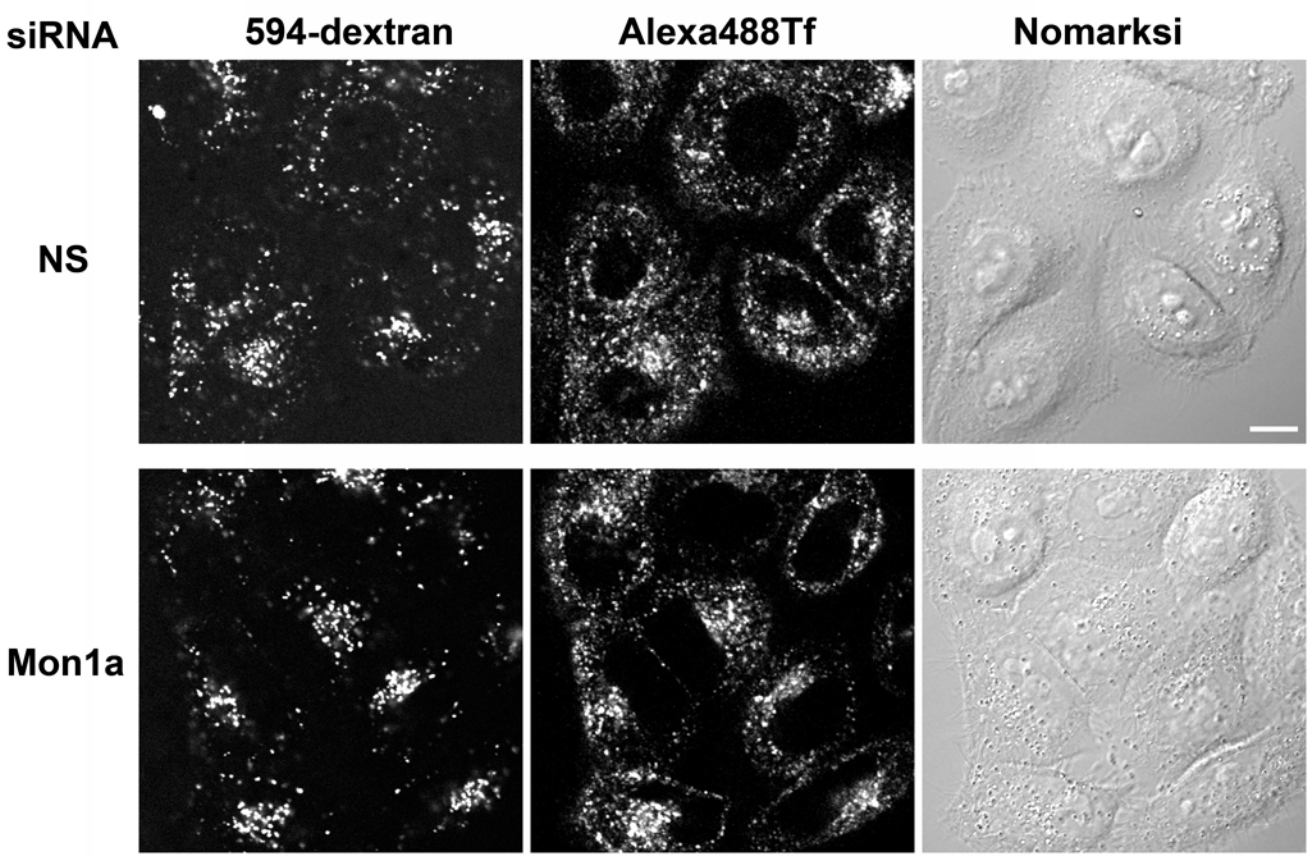
Supplemental Figure 3 Reductions in DHC1 cause Golgi fragmentation and delay movement from ER to Golgi.

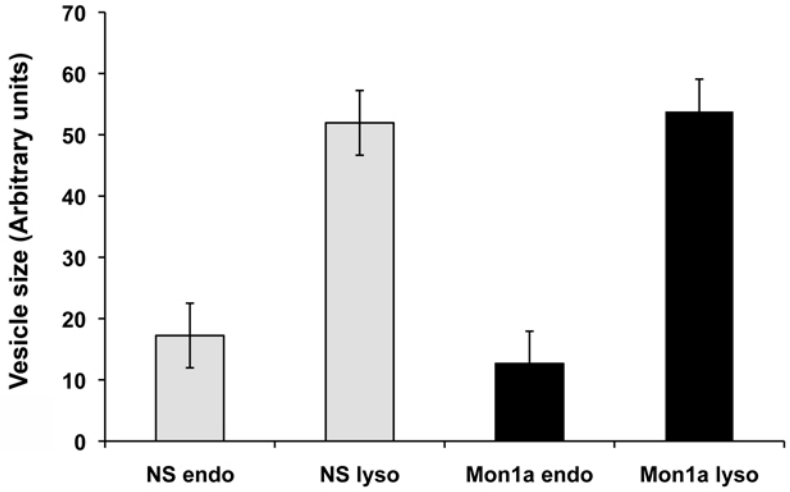
A. Mouse fibroblasts nonspecifically (NS) or DHC1 silenced for 96 h were fixed and processed for immunofluorescence using the Golgi marker Giantin and the Golgi tethering molecule p115. Images were captured on an Olympus FV1000 confocal microscope. Representative images from 50-100 cells are shown (n=3). Scale bar = 10 μm. B. HeLa cells nonspecifically (NS) or DHC1 silenced for 48 h were transfected with ts045VSVG-GFP and grown at 39.5ºC for 24 h. Cells were shifted to methionine-depleted DMEM for two h at 39.5ºC. Cells were then pulsed with ^{35}S-methionine for 30 min at 39.5ºC and shifted to 32ºC for 30 min. Cells were lysed in 1% Triton X-100/0.15 M NaCl/10 mM Tris-HCl pH7.2. Lysates were immunoprecipitated for GFP, denatured and incubated with or without Endo H for two h and radiolabeled ts045VSVG-GFP resolved on SDS-PAGE followed by autoradiography. A representative blot is presented of n=3.

Supplemental Figure 4 Sec31 is recruited at ER exit sites in Mon1a or DHC1 silenced cells.

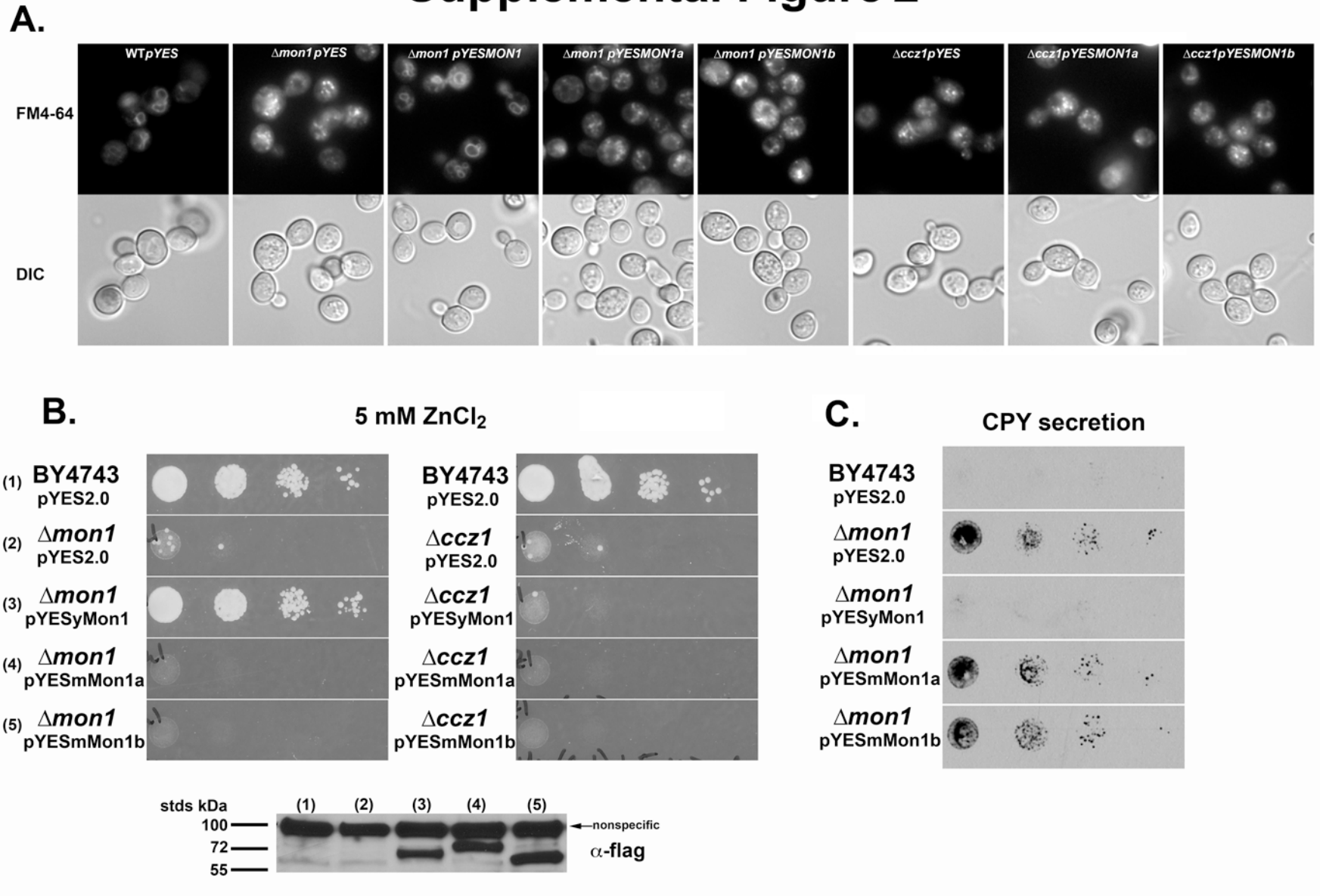
HeLa cells stably expressing GalNAc-T2-GFP were silenced with nonspecific, Mon1a or DHC1 specific oligonucleotides for 72-96 h. Cells were then treated with BFA as in figure 1, BFA washed away and the movement of GalNAc-T2-GFP from ER to Golgi and Sec31 localization assessed by epifluorescence microscopy. Representative images are shown from n=2 experiments with over 50 cells per sample examined per experiment. Scale bar = 10 μm.

Supplementary Figure 1

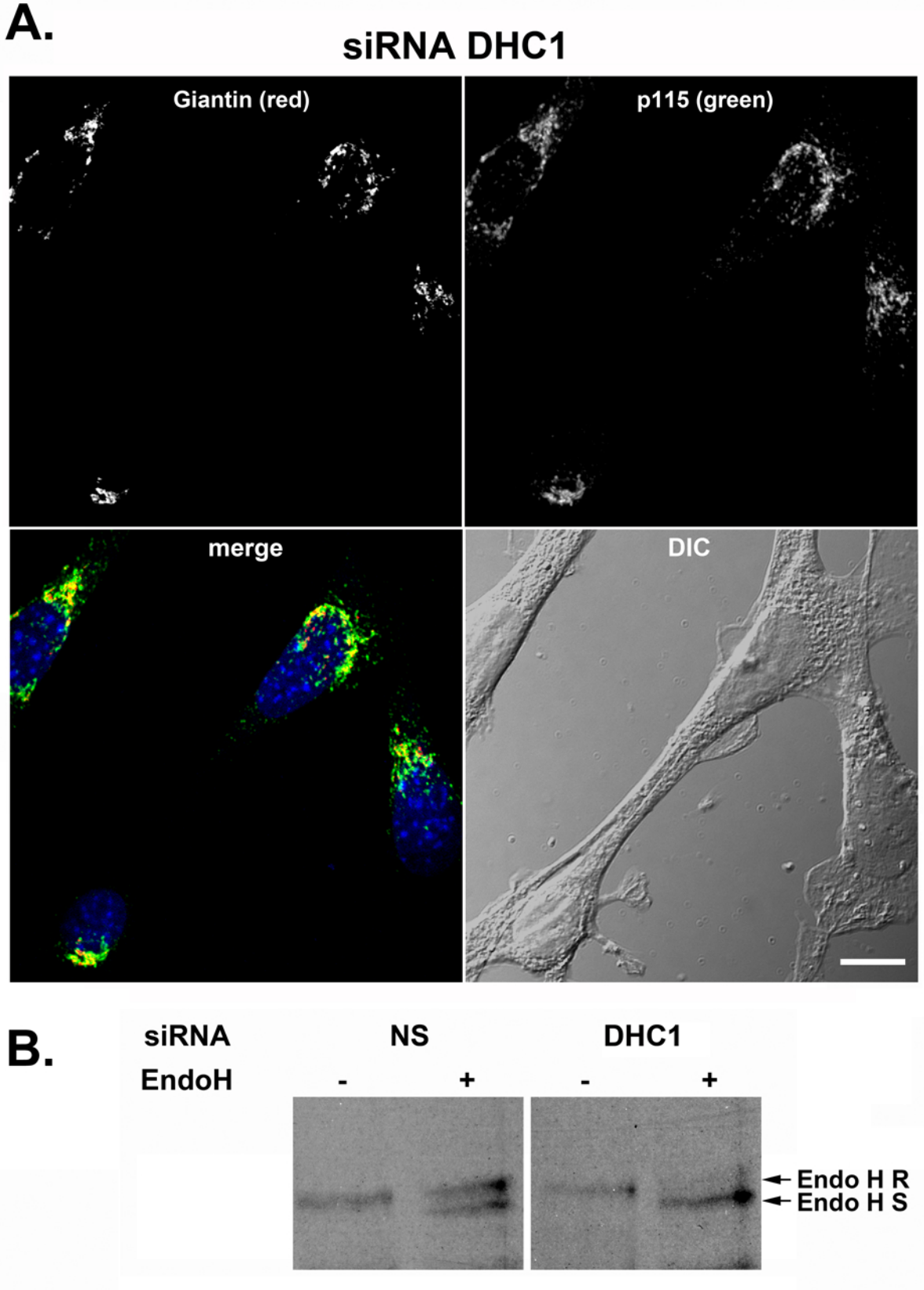




Supplemental Figure 2



Supplemental Figure 3



Supplemental Figure 4