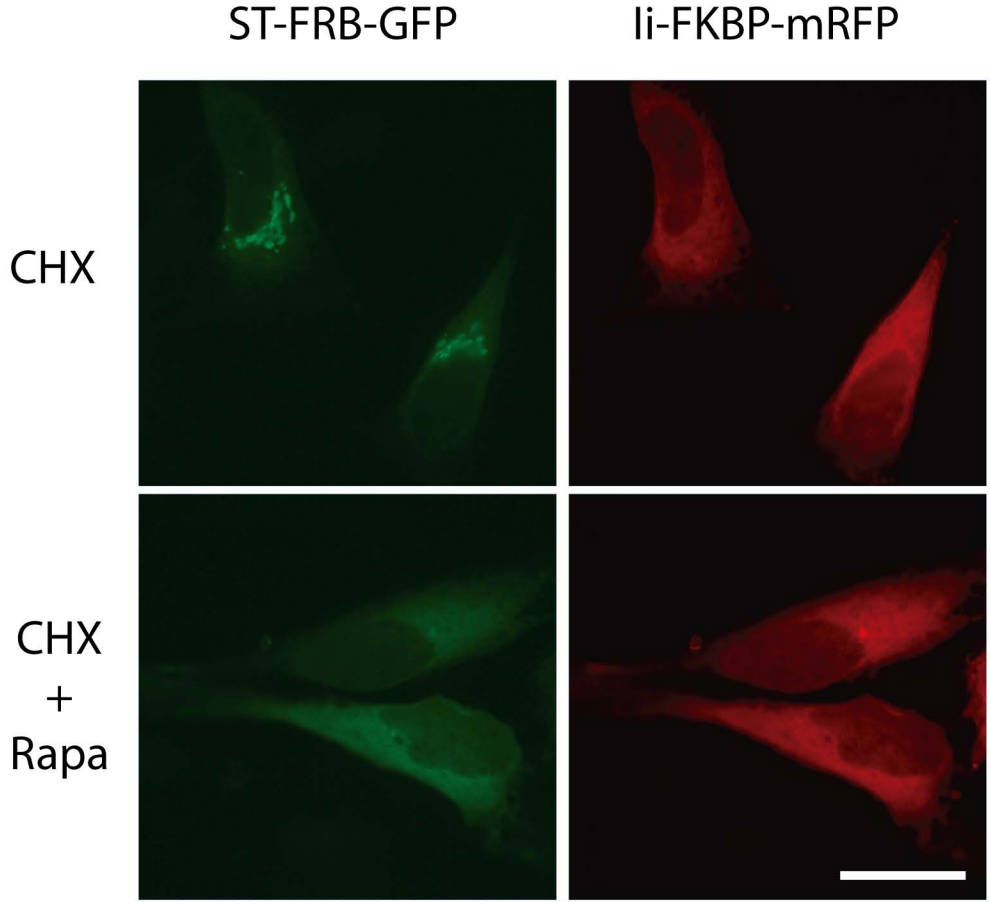
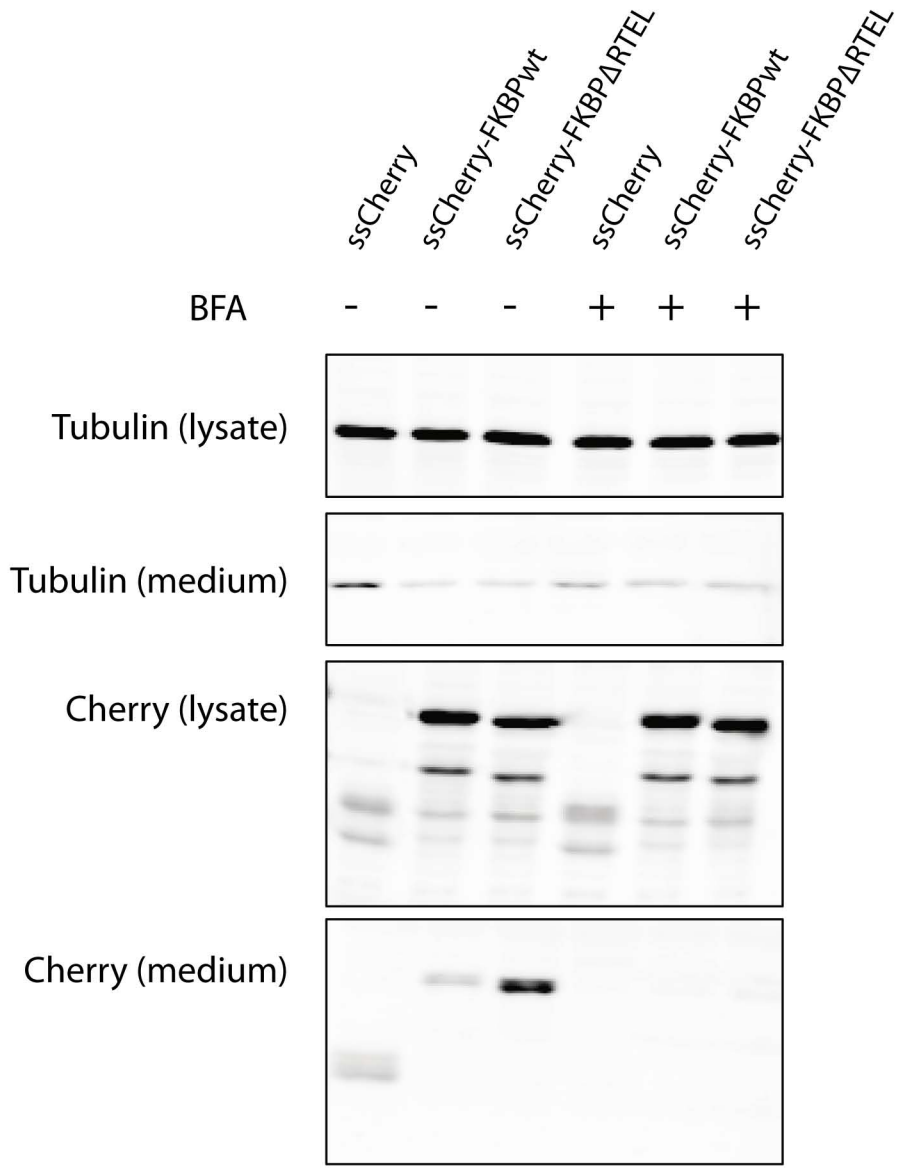
Supplemental Materials

Molecular Biology of the Cell

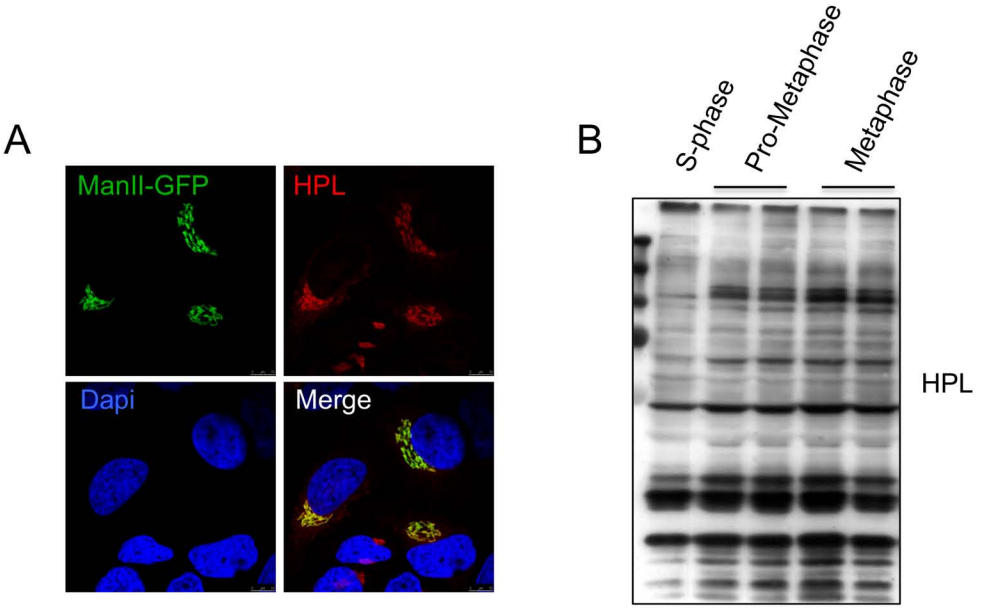
Villeneuve et al.



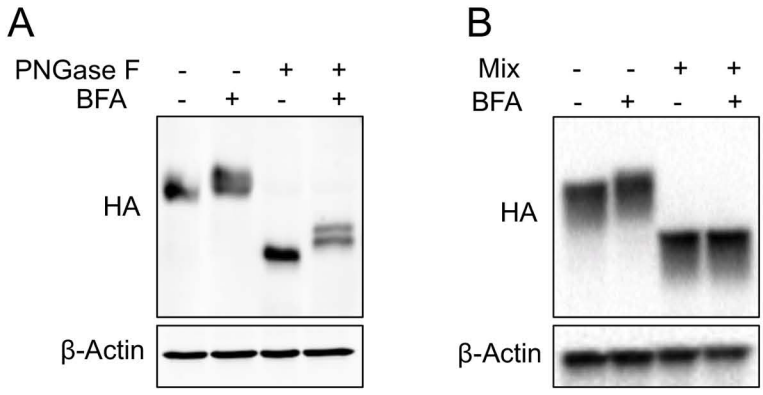
Supplemental Figure 1: B1 HeLa cells were cotransfected with ST-FRB-GFP and li-FKBP-mRFP, treated with CHX only (control) or CHX and rapamycin and fixed with PFA after 4 h. GFP and mRFP was visualized by epifluorescence (scale bar is 25 µm).



Supplemental figure 2: HeLa cells were transfected with ssCherry, ssCherry-FKBP13wt or ssCherry-FKBP13ΔRTEL. After 16 h, the medium was washed and substituted with OptiMEM without serum. BFA was added to some wells (1 µg/mL) to stop secretion. After 6 h, medium was collected, cleared by centrifugation and precipitated with trichloroacetic acid. Cell lysates and medium precipitates were loaded on acrylamide gels and western blotted with anti-Cherry and anti-tubulin antibodies.



Supplemental figure 3: (A) HeLa cells expressing ManII-GFP grown on coverslip were fixed and processed for immunofluorescence microscopy with Alexa 647-conjugated HPL and DAPI (scale bar is 10 μm). (B) Lysate from cells synchronized in S-phase, pro-metaphase and metaphase were analyzed by western blotting with HRP-conjugated HPL to observe possible changes in overall O-glycosylation.



Supplemental figure 4: HeLa cells were transfected with Ii-FRB-HA plasmid and synchronized in metaphase (double thymidine block + nocodazole + MG132 with or without BFA). Then total cell lysates were treated with or without PNGase F (A) and protein deglycosylation mix supplemented with additional exoglycosidases (B) and analyzed by western blotting with an anti-HA and an anti-β-actin antibody, respectively.