Supplemental Figures

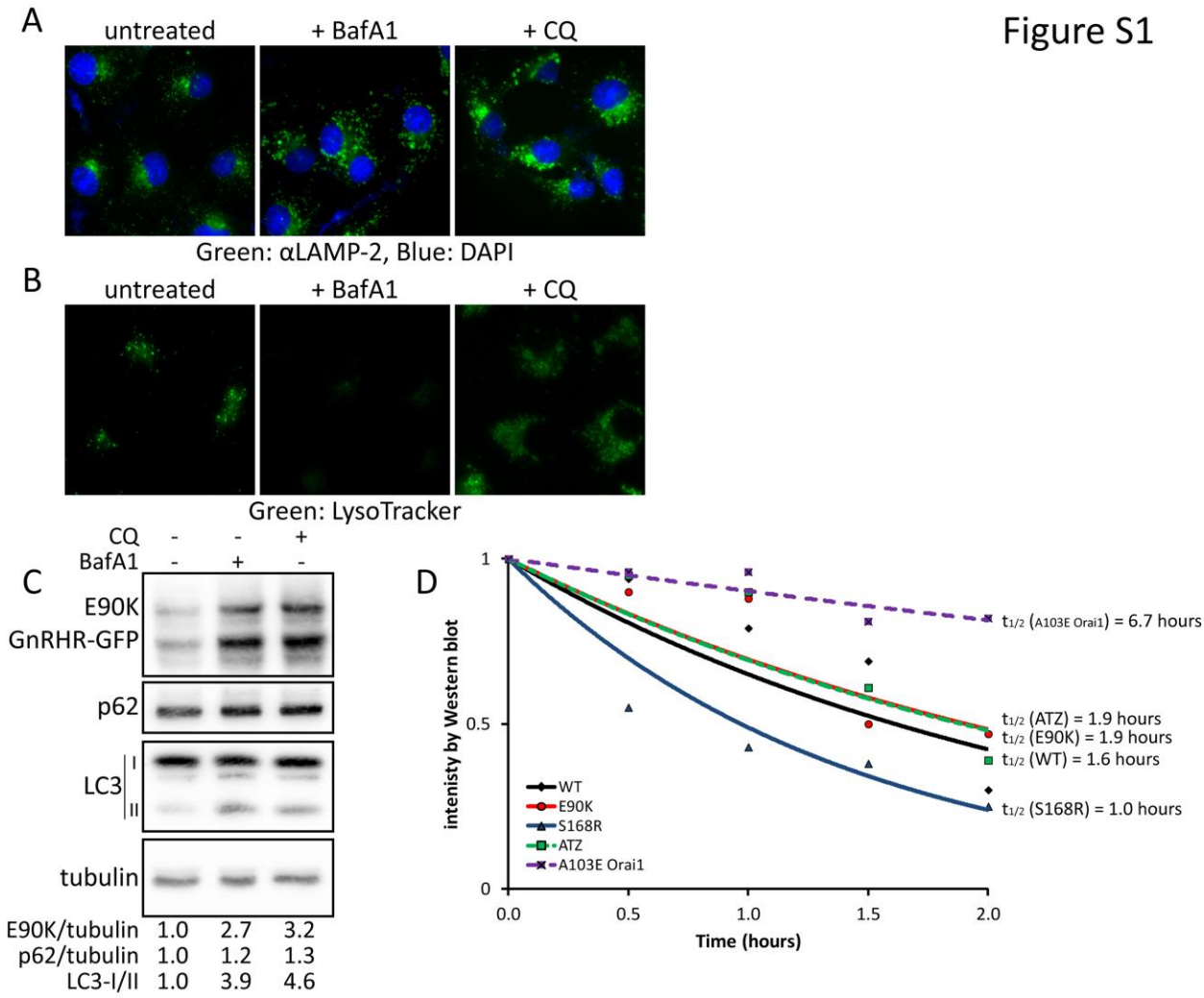

Figure S1, related to Figure 1. Bafilomycin A1 and Chloroquine both inhibit autophagy at a late-stage. **(A)** Effects of the late-stage autophagy inhibitors on lysosome morphology. Cos-7 cells were treated for 4 hours with Bafilomycin A1 (BafA1; 100nM) or chloroquine (CQ; 15μM) prior to fixation. Images are of cells immunostained for the lysosome marker LAMP2 (Green) and DAPI (Blue). Both chemicals increase the size of lysosomes so inhibition of late stage autophagy occurs. **(B)** Effects of BafA1 and CQ on lysosome acidity and morphology determined with LysoTracker (Green) and DAPI (Blue). LysoTracker puncta decrease in brightness and increase in size upon treatment with either chemical, which is consistent with inhibition of lysosome function through deacidification. **(C)** Effects of BafA1 and CQ on steady-state levels of E90K and the autophagy markers p62 and LC3. Both chemicals inhibited a late stage of autophagy as they caused an increase in levels of processed LC3II and p62. **(D)** Quantitation of ERAD substrate levels for the panel proteins represented Figure 1C. Curves shown are best-fit exponential regressions from which the half-lives ($t_{1/2}$) were calculated.

Figure S2

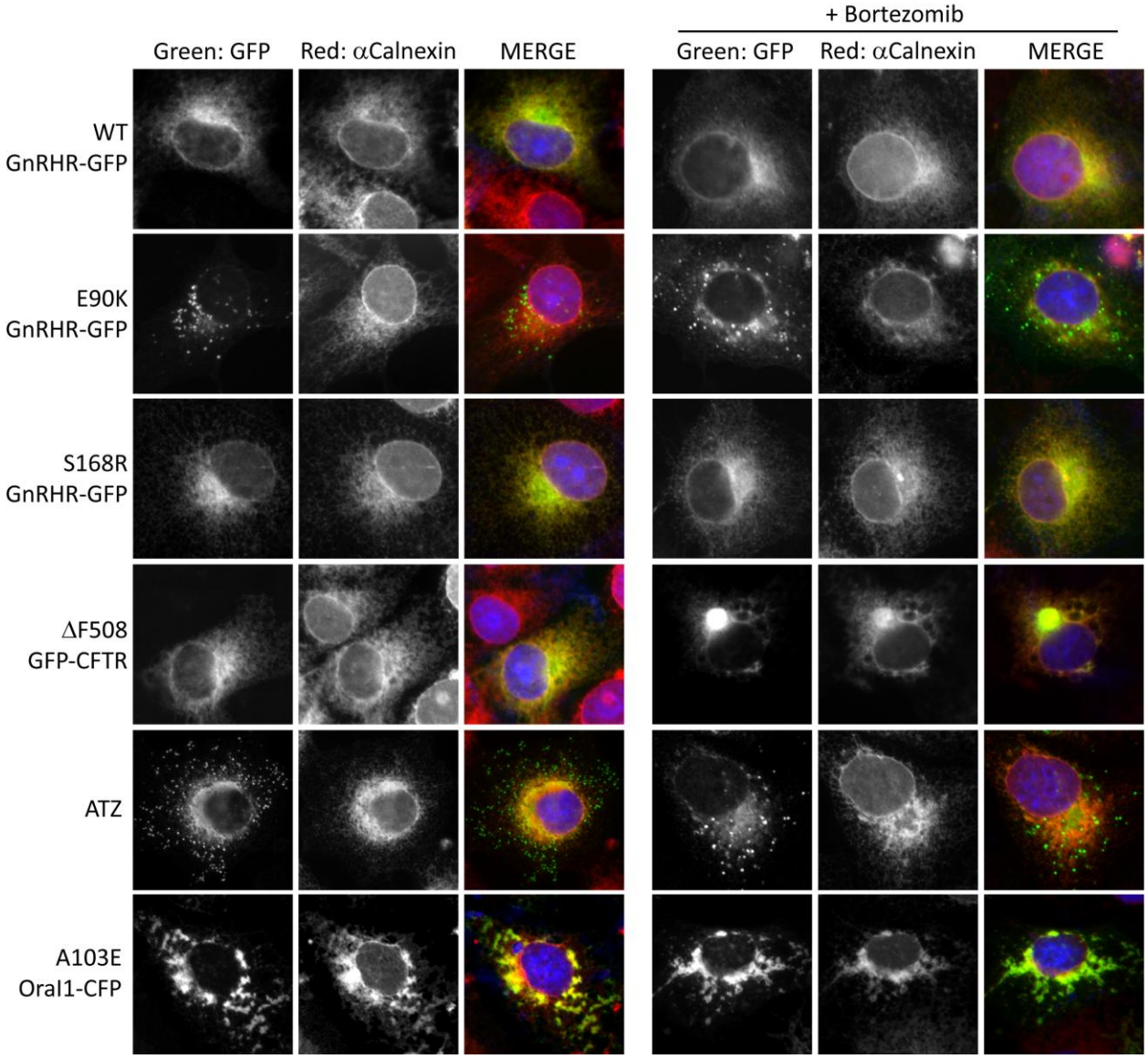

Figure S2, related to Figure 2. Fluorescence analysis of disease protein localization in fixed COS-7 cells. Individual channels from microscopy in Figure 2b are shown. The fluorescence pattern of the indicated GFP fusion protein was detected the green channel. ATZ (green) was detected by immunofluorescence, and so was the ER marker calnexin (red). Nuclei were stained with DAPI (blue).

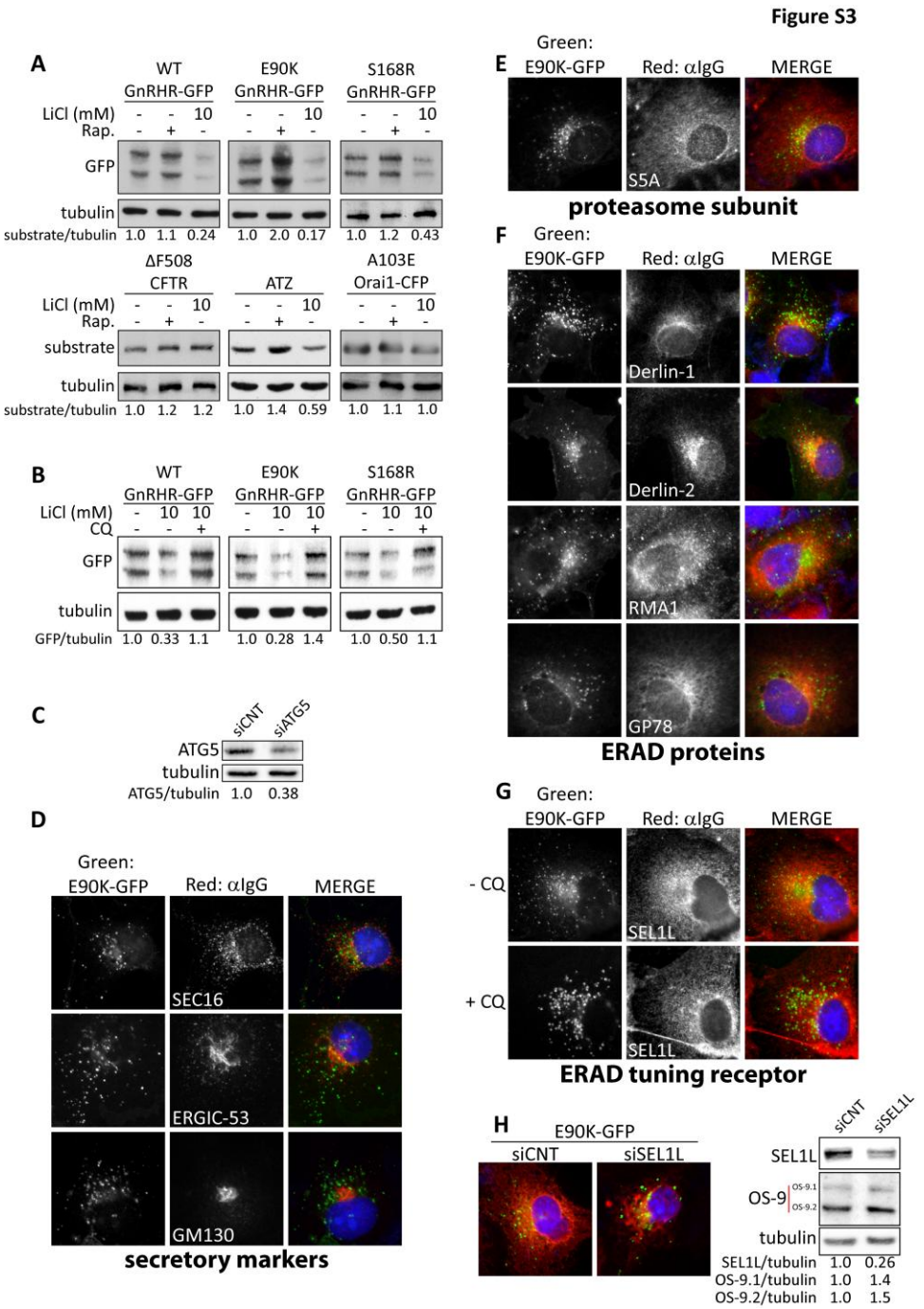

Figure S3, related to Figure 3. E90K GnRHR-GFP puncta do not colocalize with markers of the secretory pathway, proteasome, ER membrane, or ERAD tuning vesicles. (A) Effects of 500nM rapamycin and 10mM LiCl on ERQC substrate levels. Cos-7 cells were treated with chemicals for 18 hours prior to lysis and western blotting. **(B)** Effects of LiCl and CQ on E90K GnRHR-GFP levels. Cos-7 cells were treated for 12 hours with 10mM LiCl and/or 15μM CQ prior to lysis and western blotting. **(C)** ATG5 levels are decreased 62% by siATG5, as seen by western blot. **(D)** E90K GnRHR-GFP puncta do not colocalize with markers of the secretory pathway. **(E)** E90K GnRHR-GFP puncta do not colocalize with the S5A proteasome subunit. **(F)** E90K GnRHR-GFP puncta do not colocalize with indicated ER membrane proteins that function in ERAD (Red). **(G)** E90K GnRHR-GFP puncta do not colocalize with the ERAD

tuning vesicle receptor, SEL1L. **(H)** KD of the ERAD tuning vesicle receptor SEL1L with siRNA does not inhibit E90K puncta formation. Fluorescence micrographs show that in siSEL1L Cos-7 cells, E90K puncta still form. Level of knockdown is shown by western blot. In D-G the signal for E90K GnRHR-GFP in transiently transfected COS-7 cells is green and the respective marker proteins are detected by immunostaining (red) and DAPI is blue.

Figure S4

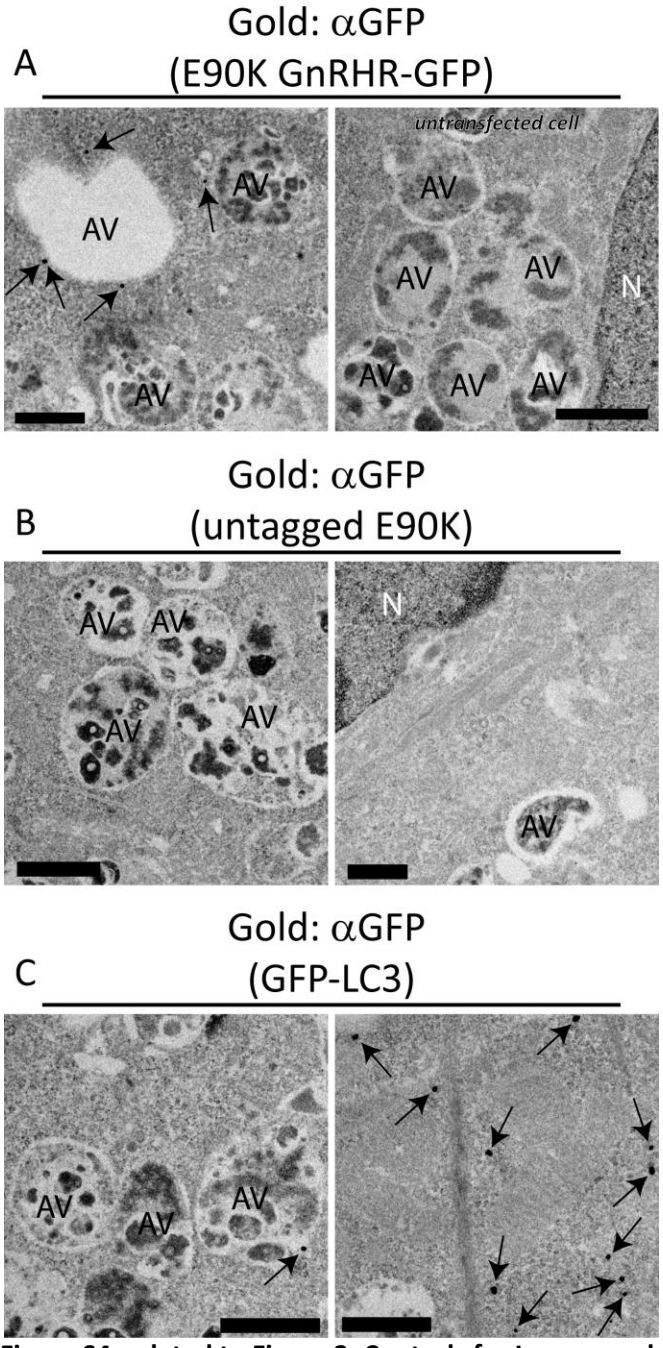

Figure S4, related to Figure 3. Controls for Immunogold TEM of E90K GnRHR-GFP. Immunogold transmission electron microscopy (TEM) of Cos-7 cells. Cell transfected with: **(A)** E90K GnRHR-GFP, **(B)** untagged E90K GnRHR, **(C)** GFP-LC3. Immunogold staining was performed against the GFP tag and gold particles are indicated with arrows. Mitochondria (M), endoplasmic reticulum (ER), nuclei (N), and autophagic vesicles (AV) are labeled. Scale bar = 1μM.

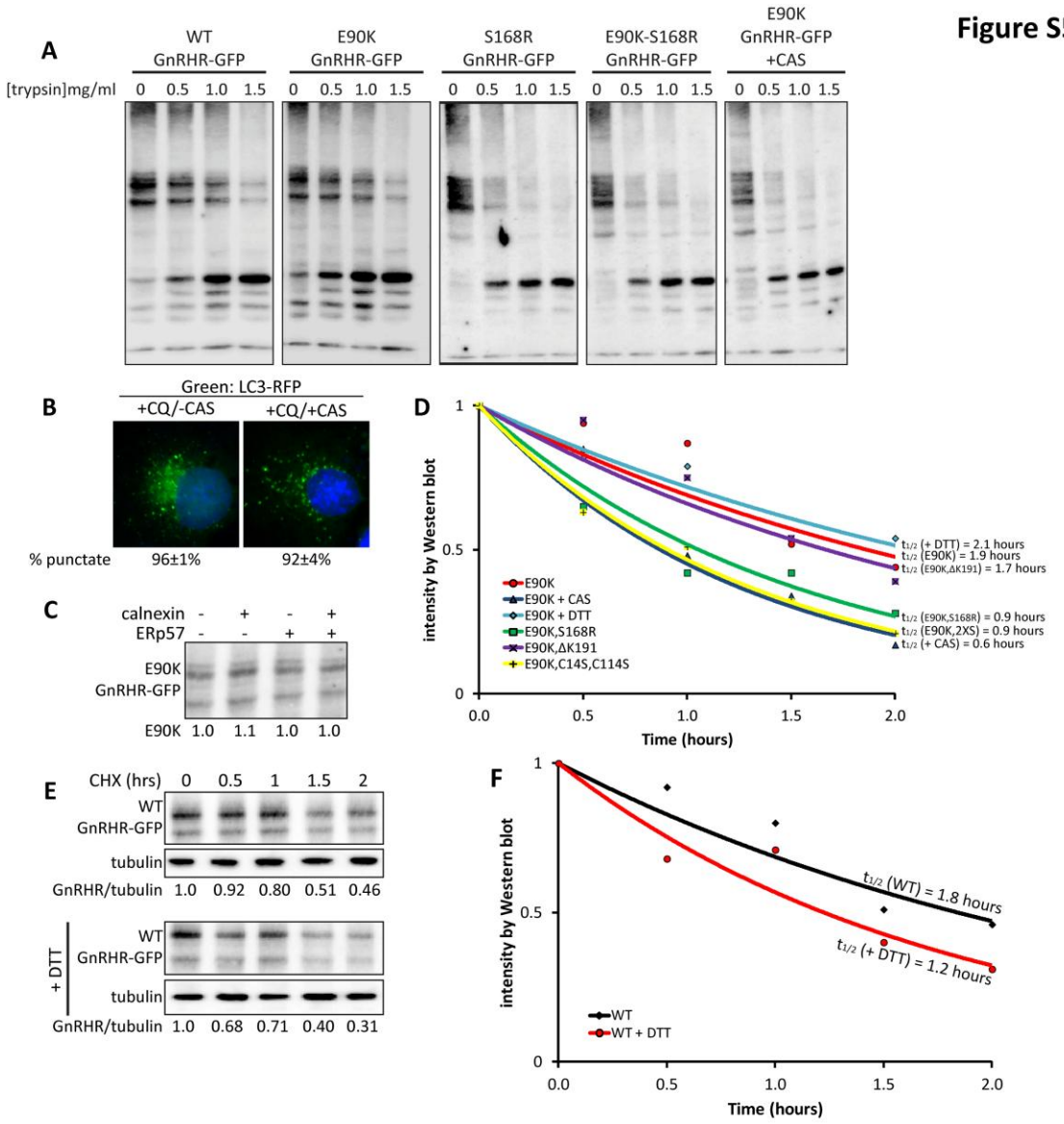

Figure S5, related to Figure 5. Effects of modulating GnRHR folding on the conformation of E90K. (A) GnRHR mutants show differential resistance to digestion with trypsin. Cos-7 cells transiently transfected with GnRHR-GFP were lysed in TBS-triton X-100 and treated with increasing concentrations of trypsin for 10 minutes at 4˚C. Western blots for anti-GFP are shown. **(B)** Castanospermine (CAS; 5mM; 4hours) does not inhibit LC3-RFP puncta formation. Fluorescence micrographs of cells expressing LC3-RFP (shown in green) in the presence and absence of CAS are shown. DAPI is blue. Cells were scored for the presence of puncta and quantification shown is the average of N=3. **(C)** Overexpression of Erp57 and calnexin have no effect on steady-state levels of E90K. Western blots of lysates from Cos-7 cells transiently transfected with E90K, Erp57, and/or calnexin are shown. **(D)** Graph showing the levels of ERQC substrate proteins calculated from the cycloheximide (CHX) chase experiments in Figure 5C. Curves shown are best-fit exponential regressions from which the half-lives ($t_{1/2}$) were calculated. **(E)** The effect of 2mM DTT on the half-life of WT. Cos-7 cells were treated with 10µg/ml CHX at t=0. **(F)** Graph showing the levels of protein calculated from the CHX chase experiments from panel D. Curves shown are best-fit exponential regressions from which the half-lives ($t_{1/2}$) were calculated.

Figure S6

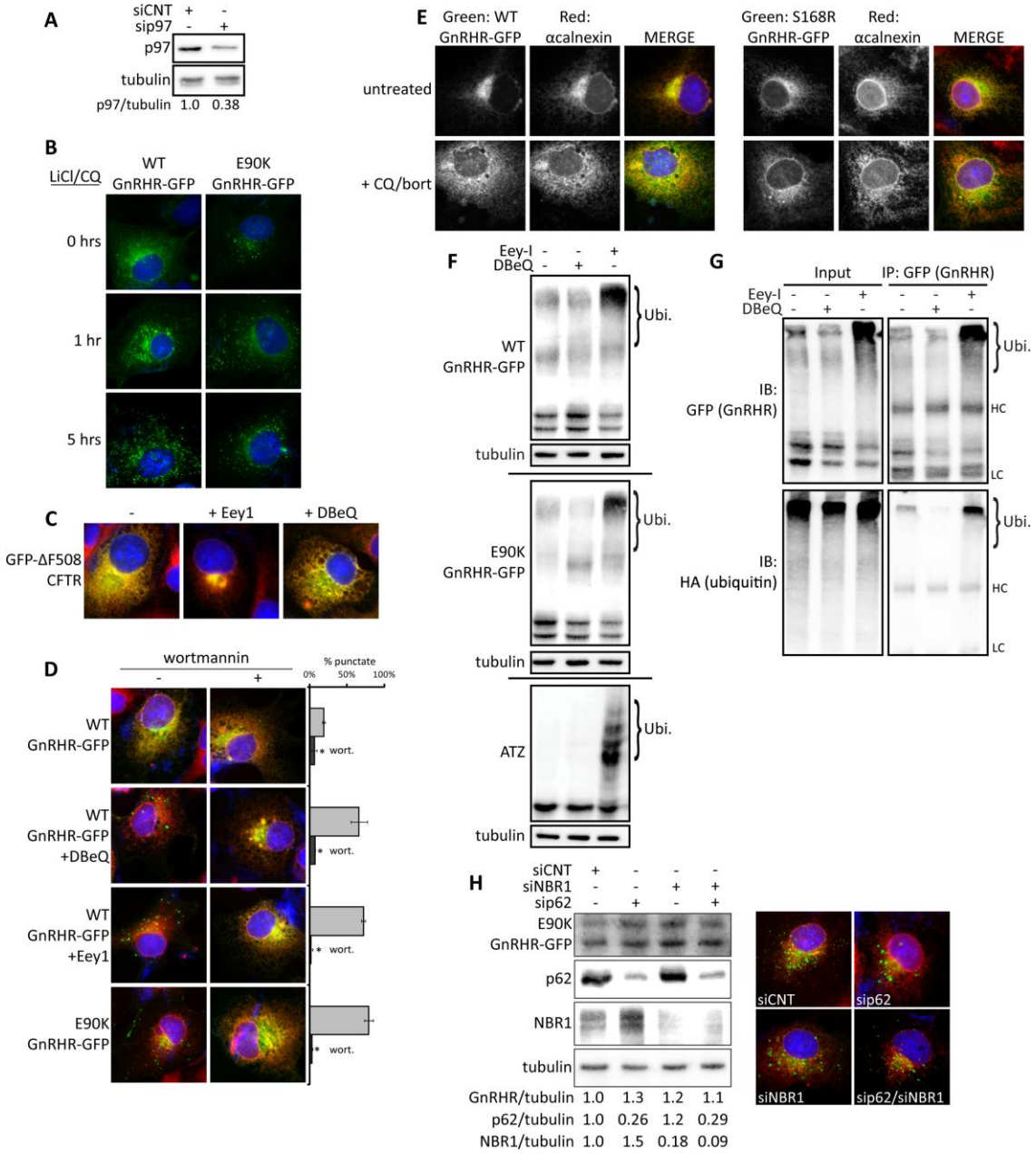

Figure S6, related to Figure 6. Knockdown of ubiquitin-binding autophagy cargo selectors does not hinder E90K puncta formation (A) Levels of p97 are reduced by 62% after treatment of cells with p97 siRNA **(B)** LiCl promotes accumulation of WT GnRHR-GFP in autophagic puncta. Fluorescence micrographs of cells Cos-7 cells transiently transfected with GnRHR-GFP and treated with 10mM LiCl and 15μM chloroquine (CQ) at time = 0 are shown. Nuclei were stained with DAPI (blue). **(C)** The effects of Eeyarstatin-1 (Eey1) and DBeQ on the localization of GFP-ΔF508 CFTR. Cos-7 cells were treated with 15μM Eey1 or DBeQ for 4 hours prior to fixation and imaging by fluorescence microscopy. Cells were immunostained for calnexin (Red) and nuclei were stained with DAPI (Blue). **(D)** The effects of wortmannin on p97-inhibitor induced GnRHR-GFP puncta. Fluorescence micrographs of Cos-7 cells that

were treated with 15μM DBeQ or Eey1, and 200nM wortmannin, for 4 hours prior to fixation. Immunostained calnexin is shown in red and nuclei are stained with DAPI (Blue). **(E)** Effects of dual inhibition of lysosome and proteasome on WT and S168R GnRHR-GFP are shown. Transiently transfected Cos-7 cell were treated with 15μM bortezomib and 10μM chloroquine for 5 hours prior to fixation. Immunostained calnexin is shown in red and nuclei are stained with DAPI (Blue). **(F)** Impact of p97 inhibitors on accumulation of GnRHR-GFP and ATZ as a high molecular weight species. Western blots for GnRHR-GFP and ATZ in the presence and absence of 15μM DBeQ or Eey1 are shown. **(G)** High molecular weight forms of GnRHR-GFP contain HA-ubiquitin. Cells were cotransfected with GnRHR-GFP and HA-ubiquitin and then treated with p97 inhibitors. GnRHR-GFP was then immune precipitated with anti-GFP and membranes of western blots were probed with GFP or HA antibody. Ubiquitinated species (Ubi), and heavy (HC) and light (LC) IgG chains are indicated.**(H)** Effects of sip62 and siNBR1 on E90K GnRHR steady-state levels and localization. Western blots of E90K GnRHR-GFP in siCNT, sip62, and siNBR1 transfected Cos-7 cells are shown. Fluorescence micrographs of siP62 or siNBR1 treated Cos-7 transfected with E90K GnRHR-GFP are shown. Nuclei are stained with DAPI (blue).

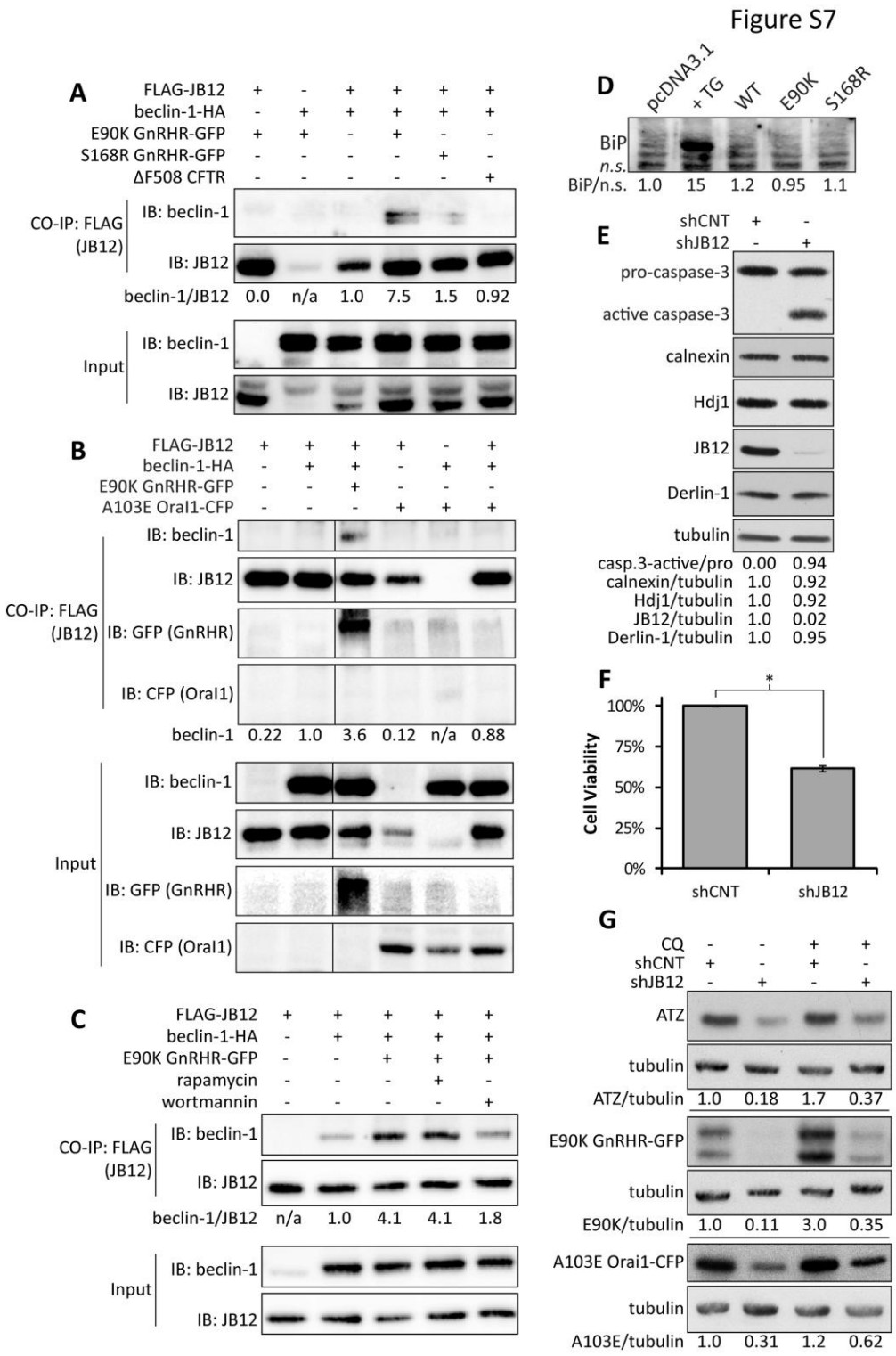

Figure S7, related to Figure 7. Specificity of interactions between DNAJB12 (JB12) and beclin-1. (A) S168R and ΔF508-CFTR does not promote association of beclin-1 with JB12. **(B)** A103E-Ora1-CFP does not promote the association of JB12 with beclin-1**. (C)** Inhibition of PI3-kinase activity prevents E90K from promoting the association of beclin-1 with JB12. For coIPs, Cos-7 cells were transiently transfected with FLAG-JB12, beclin-1-HA, E90K GnRHR-GFP, and and the indicated ERAD substrate. Native cell

extracts were made 18 hrs post-transfection. FLAG-JB12 was then immuneprecipitated using anti-FLAG agarose and membranes of western blots were probed. **(D)** Cos-7 cells were transiently transfected with GnRHR-GFP or pcDNA3.1 in the presence and absence of the ER-stress inducing chemical thapsigargin (TG). Shown are western blots for the ER-stress marker BiP. **(E)** SiRNA of JB12 induces apoptosis and reduces cell viability. Caspase-3 cleavage, and levels of JB12, calnexin, derlin-1 and Hdj1 were determined by western blots after siRNA Kd of JB12. **(F)** Reduced viability of Cos-7 cells treated with DNAJB12 siRNA was measured with the MTT assay. **(G)** Cell stress caused by JB12 knockdown dramatically reduces accumulation of E90K, ATZ and Ora1 in the presence and absence of CQ. Induction of apoptosis in response to JB12 kd appears to suppress expression of model ERQC substrates, which prevents evaluation of the requirement of JB12 in ERQC autophagy.