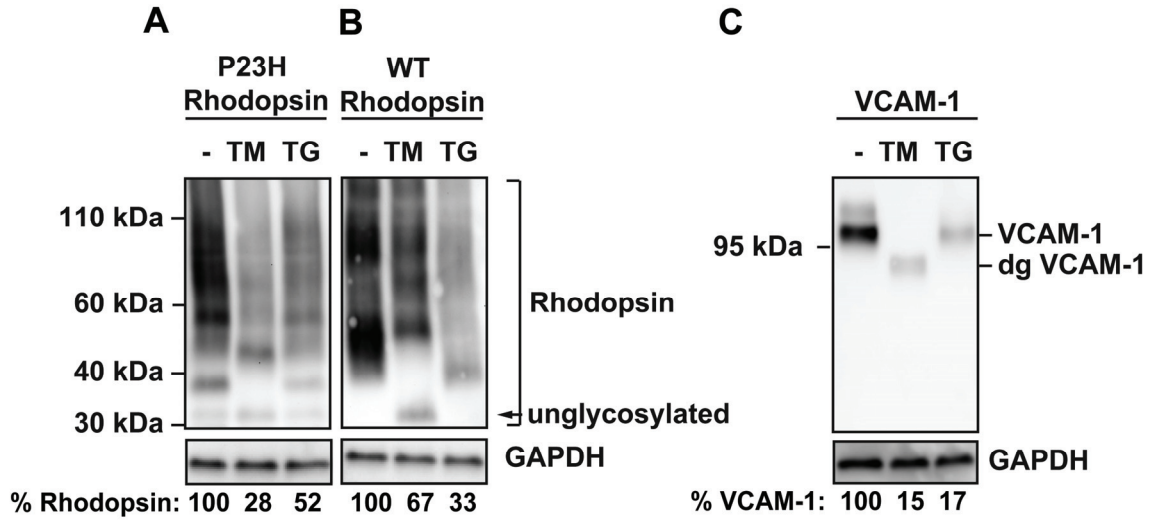
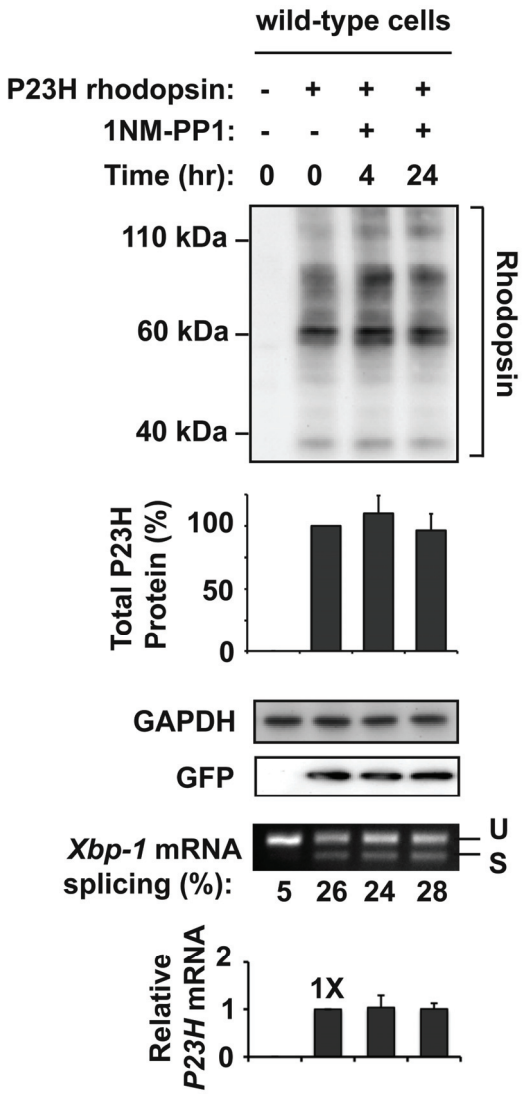
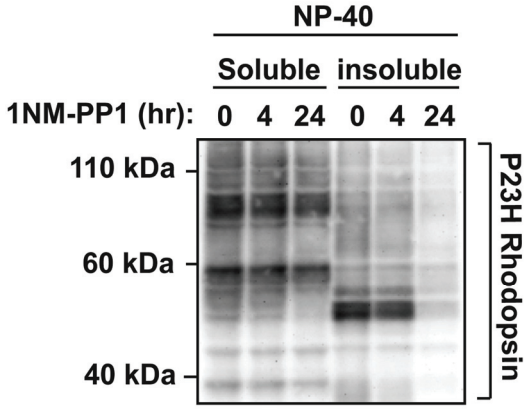


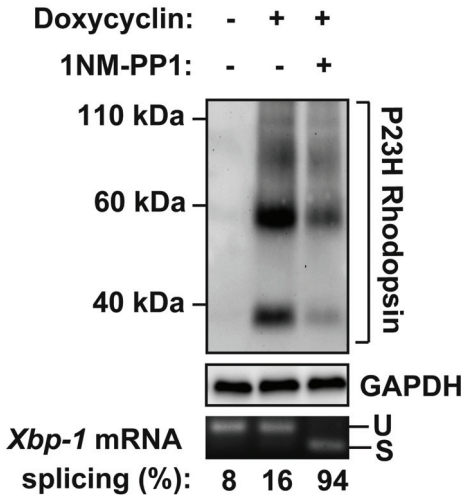
Supplemental Figure 1. Endoplasmic reticulum stress decreases levels of misfolded and wild-type membrane proteins. (A) P23H rhodopsin was expressed in HEK293 cells and tunicamycin (TM, 1 µg/mL) or thapsigargin (TG, 500 nM) was applied for 20 hours. Total P23H rhodopsin protein levels (glycosylated monomeric, dimeric, and multimeric species) were detected by immunoblotting and quantified. (B) Wild-type rhodopsin was expressed, and TM or TG was applied for 20 hours. Total wild-type rhodopsin protein levels were detected by immunoblotting and quantified. (C) Wild-type VCAM-1 was expressed, and TM or TG was applied for 20 hours. Total VCAM-1 protein levels of fully glycosylated and deglycosylated (dg) species were detected by immunoblotting and quantified. GAPDH protein levels were assessed as a protein loading control (A-C).



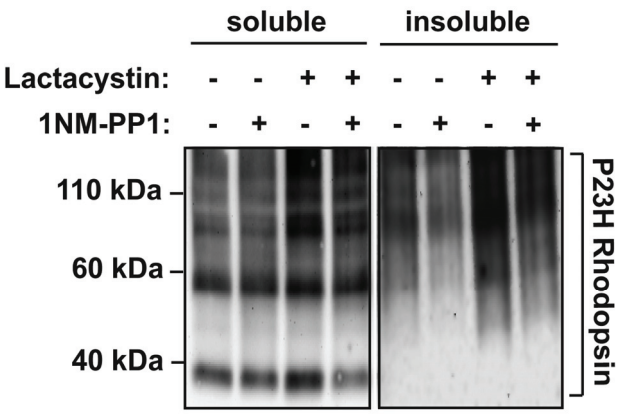
Supplemental Figure 2. Decrease of P23H rhodopsin protein levels by 1NM-PP1 requires IRE1[I642G]. P23H rhodopsin was expressed in wild-type HEK293 cells, and 1NM-PP1 (5 μM) was applied for the indicated durations. Total rhodopsin protein levels were detected by immunoblotting and quantified. GAPDH protein levels were assessed as a protein loading control. Co-transfected GFP levels were assessed to control for transfection efficiency. *Xbp-1* mRNA splicing was assessed by RT-PCR, and the amount of spliced *Xbp-1* mRNA was quantified as a percentage of the total amounts of unspliced (u) and spliced (s) amplicons. P23H rhodopsin mRNA levels were measured by real-time PCR and are expressed relative to levels in cells not treated with 1NM-PP1. Immunoblots are representative of 3 independent experiments. Error bars represent SDs from 3 experiments.



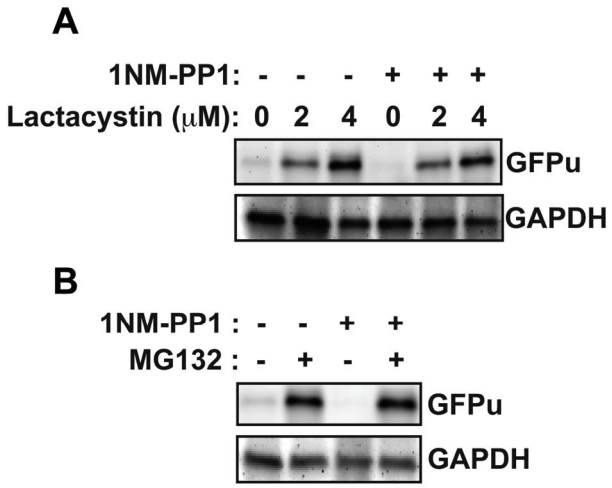
Supplemental Figure 3. P23H rhodopsin was expressed in cells bearing IRE1[I642G], and 1NM-PP1 (5 μM) was applied for the indicated time. Cell lysates were solubilized in lysis buffer containing 1% NP-40. Total rhodopsin protein levels from detergent soluble and insoluble pellet fractions were detected by immunoblotting using B630N anti-rhodopsin antibody.



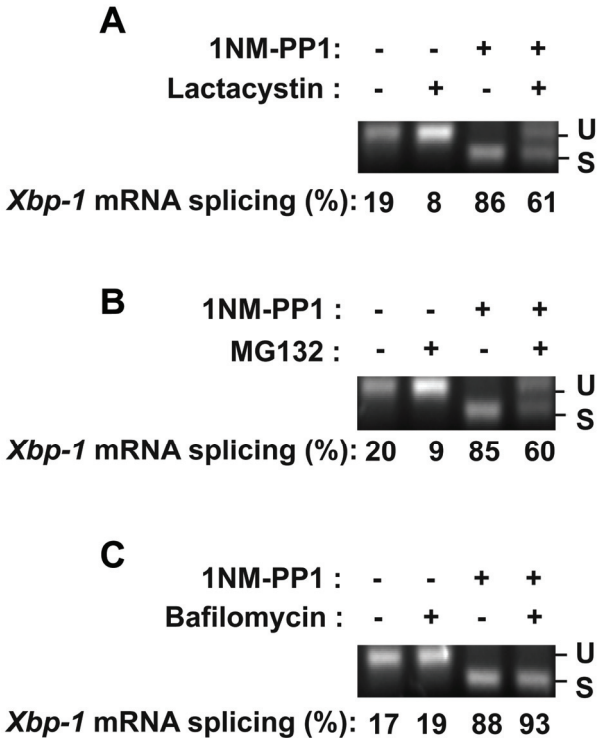
Supplemental Figure 4. Expression of P23H rhodopsin does not affect IRE1[I642G]'s ability to splice *Xbp-1* after 1NM-PP1 addition. Expression of P23H rhodopsin was induced in cells stably expressing tetracycline-inducible P23H rhodopsin and IRE1[I642G] by addition of doxycycline (1 μg/mL). Eight hours after addition of doxycycline, 1NM-PP1 (5 μM) was applied for 24 hours. Total rhodopsin protein levels were detected by immunoblotting. GAPDH protein levels were assessed as a protein loading control. *Xbp-1* mRNA splicing was assessed by RT-PCR to measure the activation of IRE1[I642G].



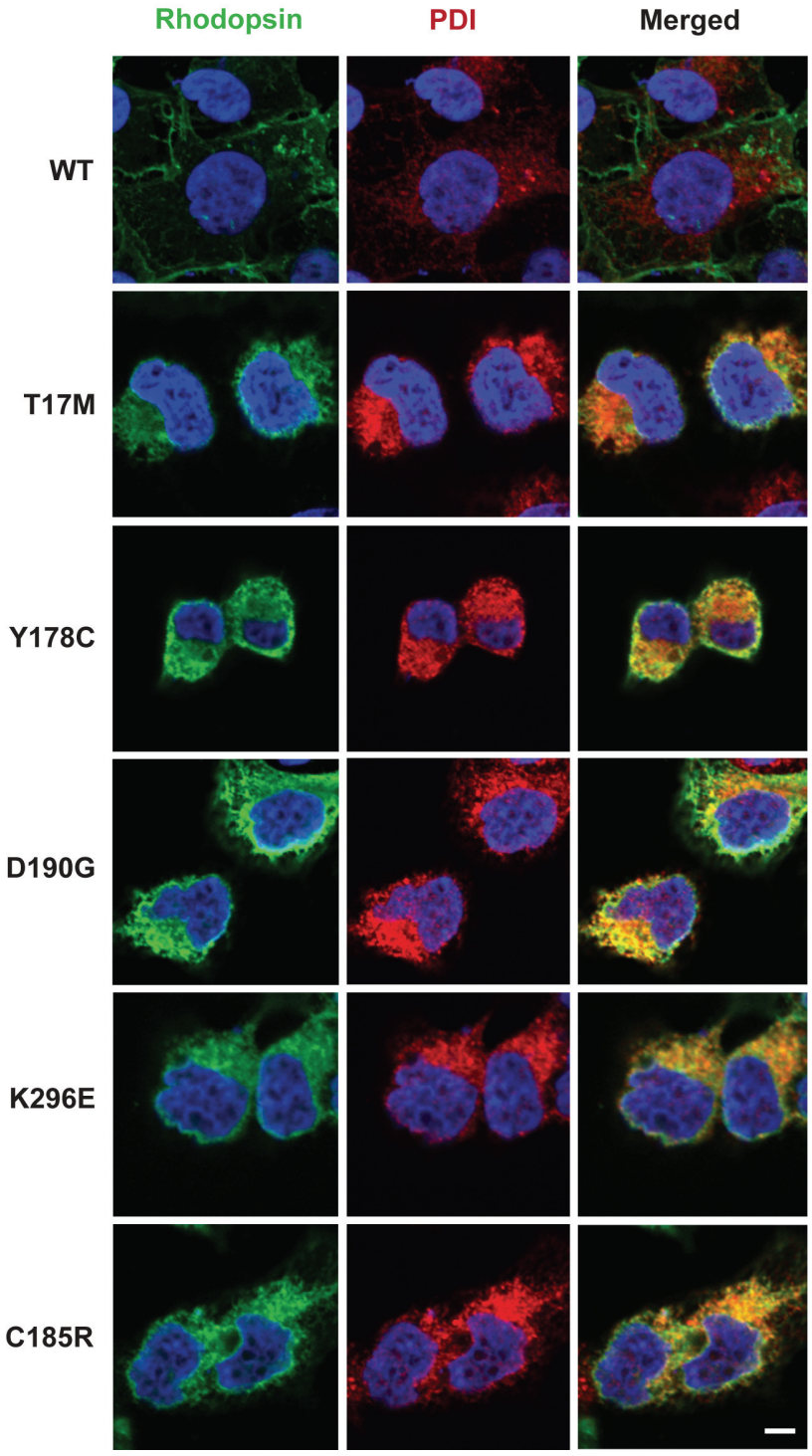
Supplemental Figure 5. Inhibiting proteasome leads to the accumulation of both soluble and insoluble P23H rhodopsin. Expression of P23H rhodopsin was induced in cells stably expressing tetracycline-inducible P23H rhodopsin and IRE1[I642G] by addition of doxycycline (1 μg/mL). 24 hours after addition of doxycycline, cells were washed and 1NM-PP1 (5 μM) and/or lactacystin (1 μM) were applied as indicated for 20 hours. Cell lysates were solubilized in lysis buffer containing 1% of NP-40. Total rhodopsin protein levels from detergent soluble and insoluble fractions were detected by immunoblotting.



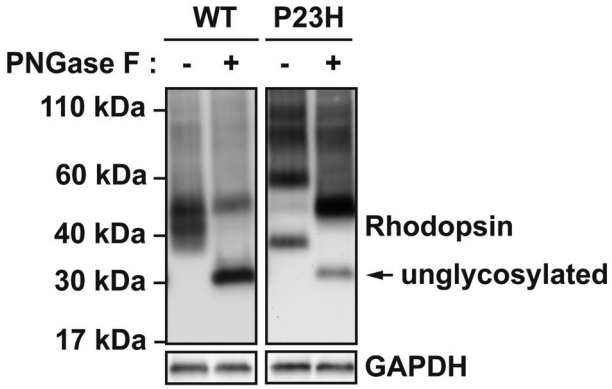
Supplemental Figure 6. Lactacystin and MG132 inhibit proteasome function. (A) GFPu was expressed in cells bearing IRE1[I642G], and lactacystin with 1NM-PP1 (5 μM) was added as indicated. (B) GFPu was expressed in cells bearing IRE1[I642G]. MG132 (0.5 μM) and 1NM-PP1 (5 μM) were applied as indicated. GAPDH protein levels were assessed as a protein loading control.



Supplemental Figure 7. 1NM-PP1 induces *Xbp-1* mRNA splicing in the presence of proteasomal or lysosomal inhibitors. Expression of P23H rhodopsin was induced in cells stably expressing tetracycline-inducible P23H rhodopsin and IRE1[I642G] by addition of doxycycline (1 μg/mL). Eight hours after addition of doxycycline, cells were washed and 1NM-PP1 (5 μM), lactacystin (1 μM), MG132 (1 μM), and bafilomycin (1μM), were applied as indicated for 20 hours. *Xbp-1* mRNA splicing was assessed by RT-PCR to assess the activation of IRE1[I642G].



Supplemental Figure 8. Wild-type or mutant T17M, Y178C, D190G, C185R, or K296E rhodopsins were expressed in HEK293 cells, and the subcellular localization of rhodopsin (green) was visualized by immunofluorescence labeling and confocal microscopy. The endoplasmic reticulum was visualized by PDI (red) immunostaining. The nucleus was visualized by DAPI staining (blue). Magnification bars, 5 μm.



Supplemental Figure 9. Identification of unglycosylated rhodopsin. Cell lysates expressing wild-type or P23H rhodopsin were treated with PNGase F as indicated and the deglycosylated species was resolved by SDS-PAGE immunoblotting analysis. GAPDH protein levels were assessed as a protein loading control.