Supplementary material

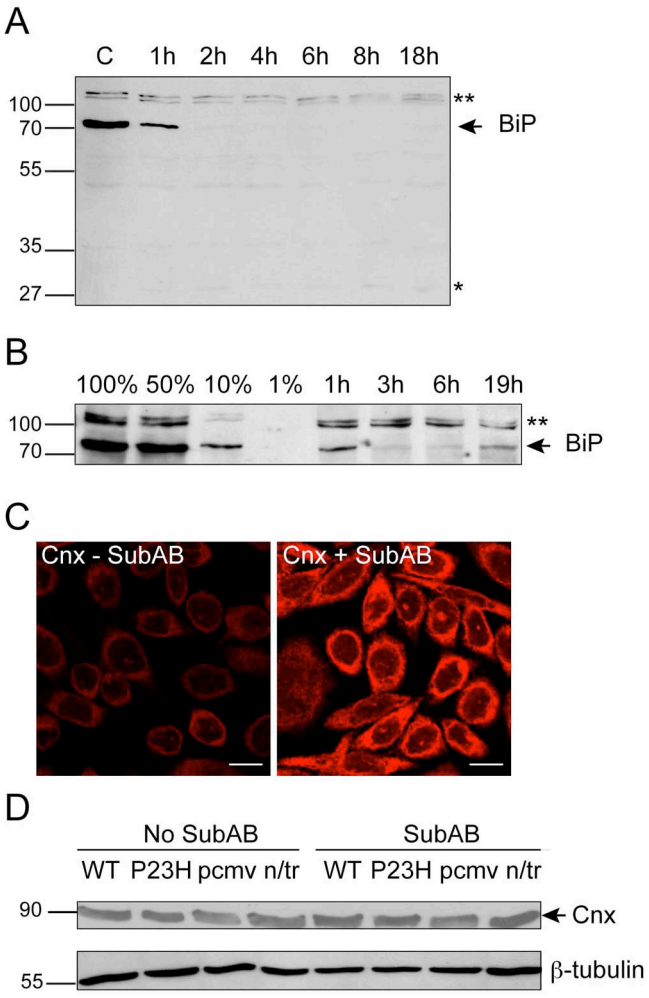

Figure S1. The effect of SubAB-mediated cleavage on BiP expression. (**A**) SK-N-SH cells were treated with 1.5 µg/ml SubAB for the times indicated (hours). The untreated control (C) cells had a prominent band at 78 kDa corresponding to BiP while each hourly SubAB treatment up to 5 hours and between 17 and 24 hours showed a reduction of this band. Treatments between 1-5 hours, the cleavage product of BiP at 28 kDa was also discernible (*). A non-specific band at approximately 100 kDa (**) confirmed equal protein loading. (**B**) Calibration curve for BiP detection by Western blotting. Untreated SK-N-SH cells were lysed in 1% Triton X-100 and serial dilutions were prepared as indicated. The levels of BiP at four time points were compared to BiP levels in serial dilutions. The levels of remaining BiP after 1, 3, 6 and 19 hour SubAB treatment were found to be between 1% and 10%. The position of molecular weight markers in kDa are indicated on the left. The non-specific band at approximately 100 kDa (**) confirmed equal protein loading. (**C**) The effect of SubAB-mediated cleavage on Cnx staining. SK-N-SH cells were treated with 1.5 µg/ml SubAB or vehicle alone for 18 hours. Cells were analyzed by confocal microscopy and scanning intensity levels between control and SubAB treated cells were kept the same. Scale bar 10 µm. (**D**) Western blot showing Cnx levels (upper panel) following SubAB treatment. WT, P23H or empty vector (pcmv) rod opsin transfected or non-transfected cells (n/tr), β-tubulin (lower panel) was blotted as a loading control. 10 µg total protein loaded for each lane.

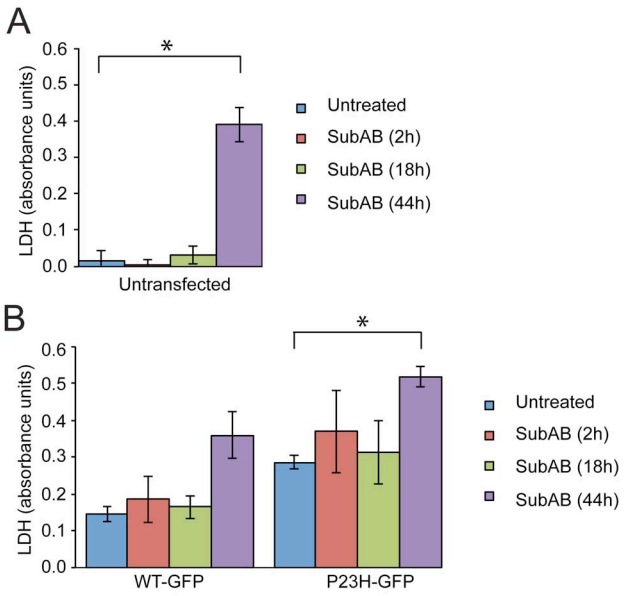

Figure S2. SubAB treatment does not cause cell death at early time points of treatment. LDH assay of untransfected (**A**) and rod opsin transfected (**B**) SK-N-SH cells treated with SubAB. Average absorbance readings for untreated cells (blue) and SubAB treated cells for 2 (red), 18 (green) and 44 (purple) hours. For transfected cells (**B**), the background cell death of GFP only transfection was subtracted from the values for WT-GFP and P23H-GFP transfections to allow for transfection related cell death. Note as previously published (Mendes and Cheetham, 2008), P23H expression alone leads to enhanced cell death. Cell death was only significant after 44 hours of SubAB treatment. Error bars are two standard errors, n=3, * $p<0.05$.

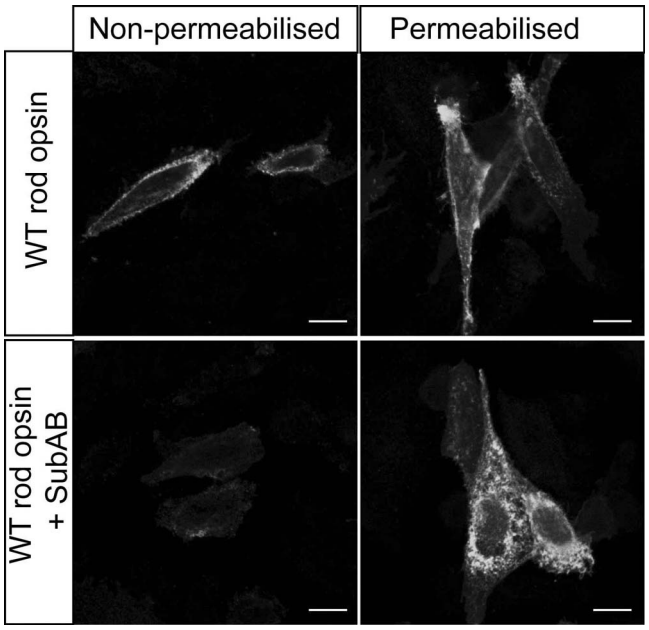

Figure S3. SubAB-mediated cleavage of BiP impairs WT rod opsin traffic. SK-N-SH cells were transfected with WT rod opsin-pMT3 and were left untreated (upper panels) or treated with 1.5 µg/ml SubAB (lower panels) for 18 hours prior to fixation with 4% PFA. Following fixation cells were either not permeabilised (left panels) or permeabilised with 0.5 % Triton (right panels), as indicated. 4D2 primary antibody (1:100) and anti-mouse Cy3 (1:100) secondary antibody was used to detect rod opsin immunoreactivity. Cells were analysed by confocal microscopy keeping the same scanning intensity levels between non-permeabilised and permeabilised control and SubAB treated cells. Scale bar 10 µm.

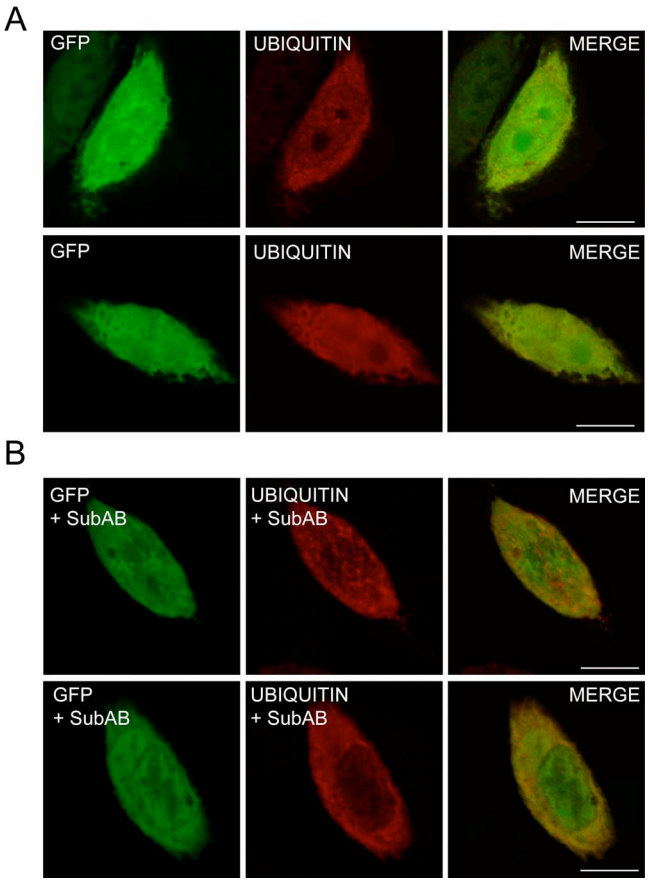

Figure S4. SubAB treatment effect on ubiquitin localization in the absence of rod opsin. Representative images of SK-N-SH cells transfected with GFP and myc-ubiquitin (upper panels) and treated with SubAB (lower panels). Two hours after transfection, cells either had a change of media or were treated with 1.5 μg/ml SubAB for 18 hours. Scale bar 10 μm.

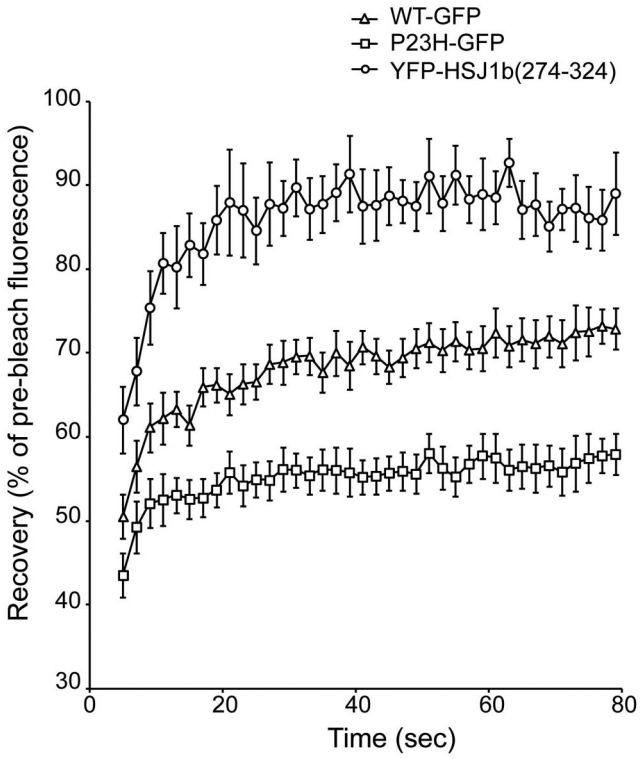

Figure S5. Relative mobilities of different ER associated proteins. Graphical representation of recovery after photobleach for YFP-HSJ1b(274-324) (circles), compared to WT rod opsin (WT-GFP) (triangles) and P23H-GFP in the absence of SubAB treatment. Fluorescence intensities for the 2x2 μm area were normalised to pre-bleach levels at 100%, error bars represent standard error, $n \geq 12$.

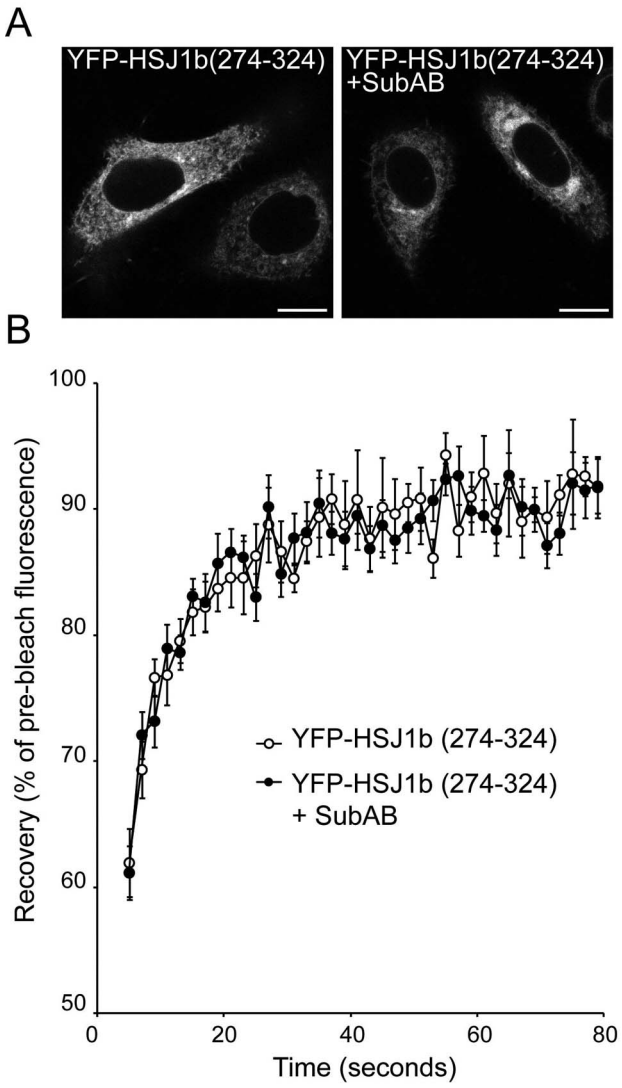

Figure S6. SubAB treatment does not affect the mobility of a protein associated with the ER cytoplasmic face. (**A**) The localization of YFP-HSJ1b(274-324) is not altered by SubAB treatment. Scale bar 10 μm. (**B**) Graphical representation of recovery after photobleach for YFP-HSJ1b(274-324) (open circles) compared to WT YFP-HSJ1b(274-324) in the presence of SubAB treatment (closed circles). Fluorescence intensities for the 2x2 μm area were normalised to pre-bleach levels at 100%, error bars represent standard error, $n \geq 12$.

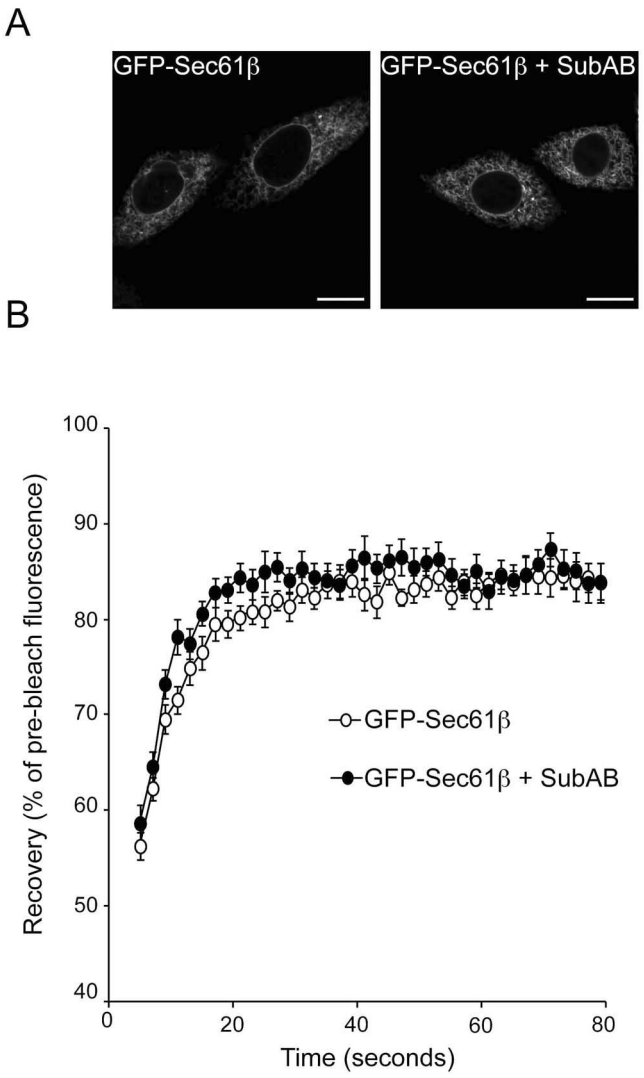

Figure S7. SubAB treatment does not affect the mobility of an ER membrane anchored protein. (**A**) The localization of GFP-Sec61β is not altered by SubAB treatment. Scale bar 10 μm. (**B**) Graphical representation of recovery after photobleach for GFP-Sec61β (open circles) compared to GFP-Sec61β in the presence of SubAB treatment (closed circles). Fluorescence intensities for the 2x2 μm area were normalised to pre-bleach levels at 100%, error bars represent standard error, $n \geq 12$.

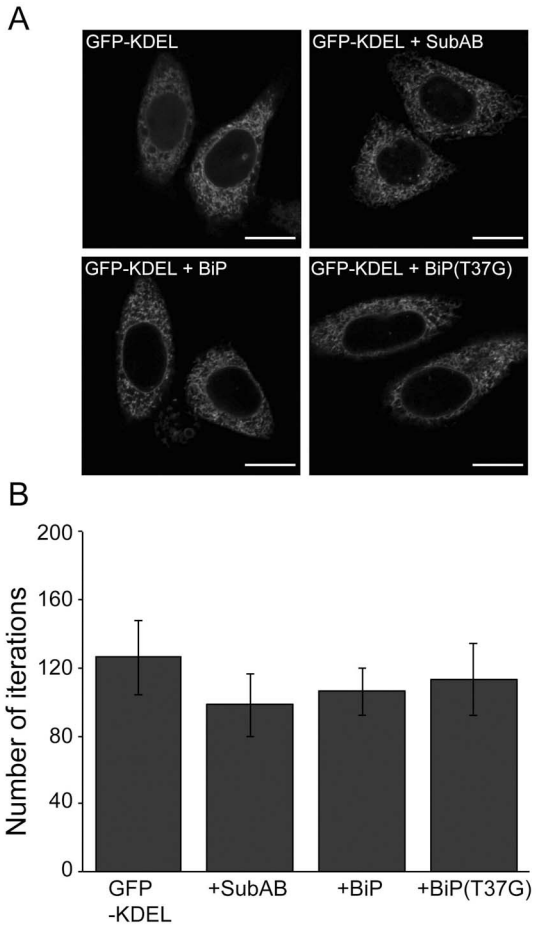

Figure S8. BiP manipulation does not affect the mobility of a soluble lumenal GFP fusion protein. (**A**) The localization of GFP-KDEL is not altered by SubAB treatment or WT-BiP or BiP(T37G) overexpression. Scale bar 10 μm. (**B**) Graphical representation of the number of 100% intensity flashes required to bleach the ER ROI intensity to 40%. Error bars represent two standard errors, $n \geq 12$.

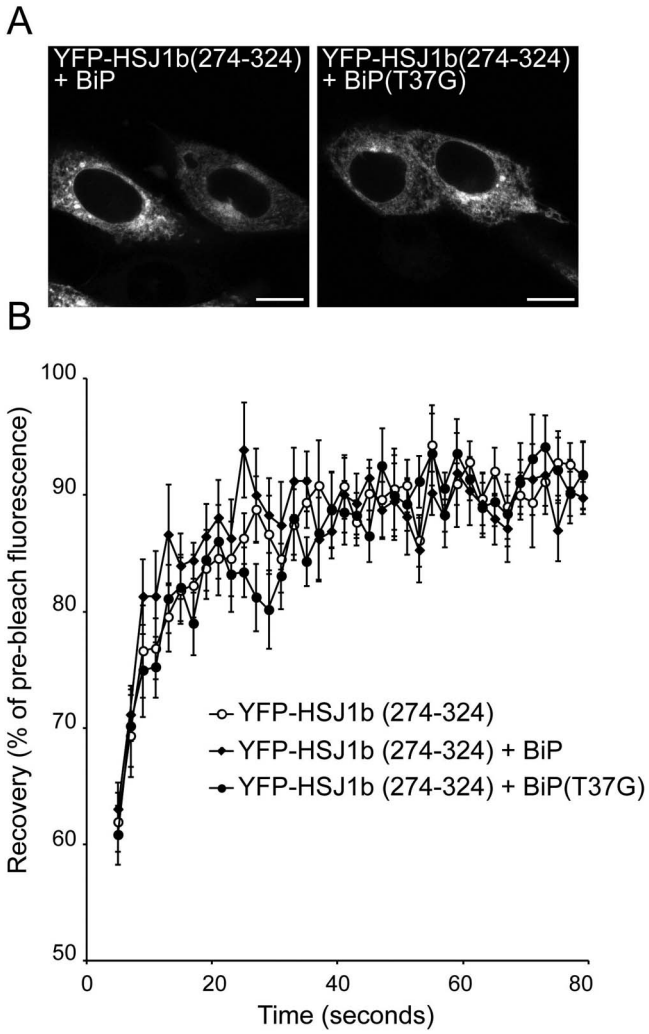

Figure S9. BiP(T37G) does not affect the mobility of a protein associated with the ER cytoplasmic face. (**A**) The localization of YFP-HSJ1b(274-324) is not altered by BiP(T37G) overexpression. Scale bar 10 μm. (**B**) Graphical representation of recovery after photobleach for YFP-HSJ1b(274-324) (open circles) compared to YFP-HSJ1b(274-324) in the presence of BiP WT (closed diamonds) or BiP(T37G) (closed circles). Fluorescence intensities for the 2x2 μm area were normalised to pre-bleach levels at 100%, error bars represent standard error, $n \geq 12$.

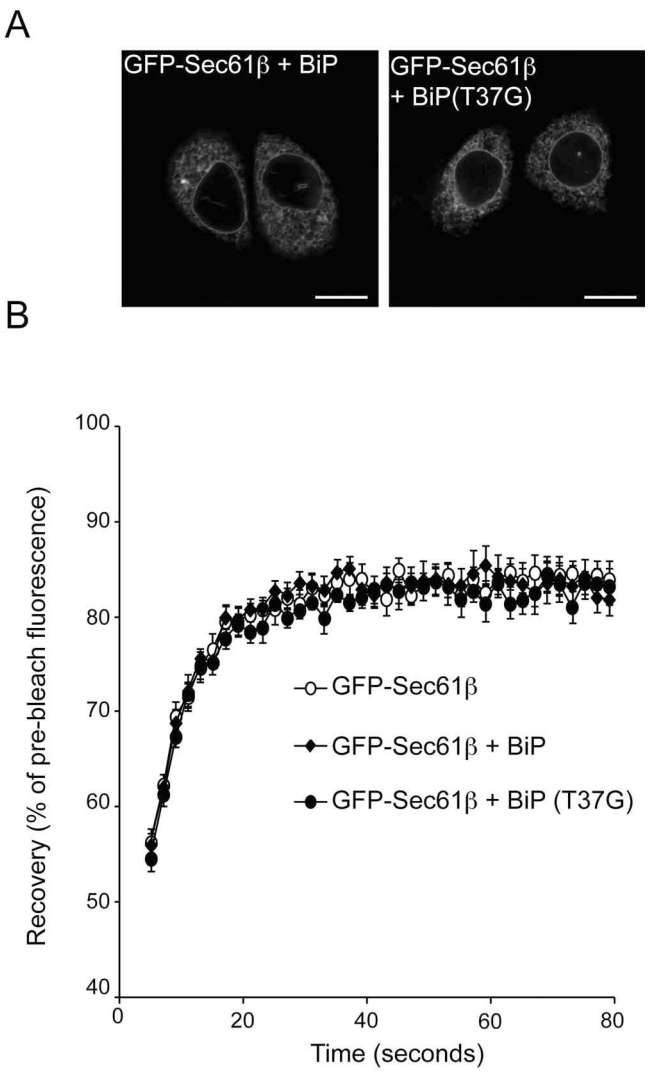

Figure S10. BiP(WT) and BiP(T37G) do not affect the mobility of the GFP-Sec61β (**A**) The localization of GFP-Sec61β is not altered by WT-BiP or BiP(T37G) overexpression. Scale bar 10 μm. (**B**) Graphical representation of recovery after photobleach for GFP-Sec61β (open circles) compared to GFP-Sec61β in the presence of BiP (WT) (closed diamonds) or BiP(T37G) (closed circles). Fluorescence intensities for the 2x2 μm area were normalised to pre-bleach levels at 100%, error bars represent standard error, $n \geq 12$.