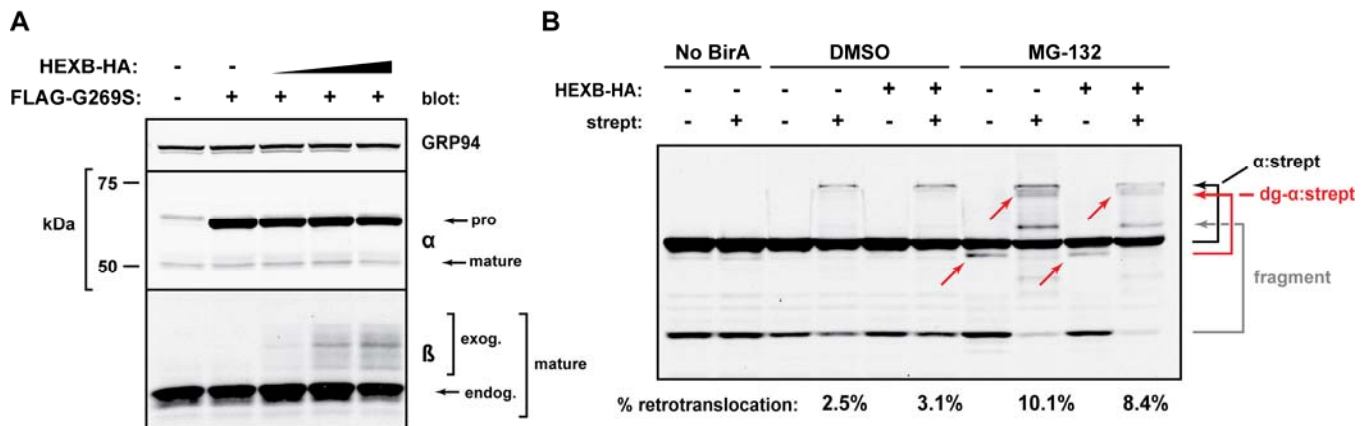


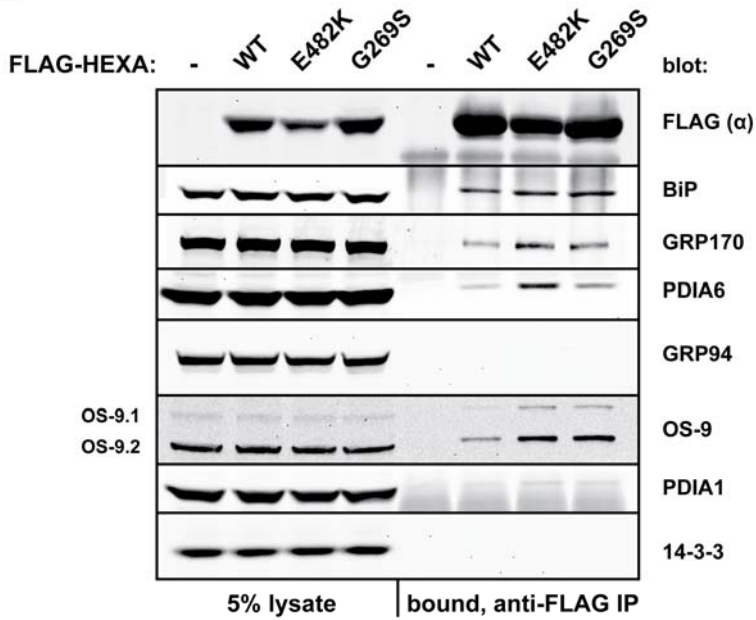
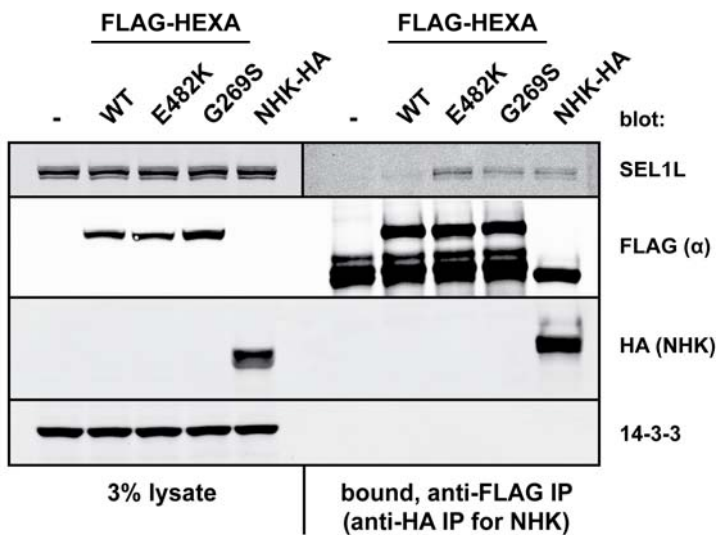
Supplemental Materials

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Supplemental Figure 1. Targeted analysis of ER quality control factors that associate with α . (A) FLAG-HEXA constructs were expressed in HEK293T cells for 20 hours, followed by immunoprecipitation with anti-FLAG agarose to observe co-IP of the indicated endogenous chaperones and ERAD factors. 14-3-3, cytosolic control to ensure stringency of the IP. (B) Same as A, except HA-tagged NHK was also expressed and precipitated with anti-HA agarose as a positive control for SEL1L association. Lysate and co-IP samples of SEL1L were analyzed independently due to the low signal in IP samples.

A**B**

Supplemental Figure 2. β expression does not improve G269S trafficking. (A) HEK293T cells were co-transfected with G269S FLAG-HEXA and either 0, 0.5, 1.5, or 3 μ g of HEXB-HA plasmid DNA. After 24 hours, cells were harvested for immunoblot analysis to determine proteolytic processing of G269S. (B) G269S-V5-BAP was expressed in HEK293T cells alone or in combination with HEXB-HA; cells were treated with biotin and either DMSO or MG-132 and harvested for determination of retrotranslocation as described in Materials and Methods and Figure 6. % retrotranslocation is indicated under the respective lanes. Arrows on the right indicate the size shifts of bands due to streptavidin binding. Small red arrows indicate where cytosolic, deglycosylated species should run on the gel. α :strept, the α -biotin-streptavidin complex; dg- α , de-glycosylated α ; fragment, a fragment observed in the cytosol during proteasomal inhibition.