Supplemental Data

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FIGURE LEGENDS

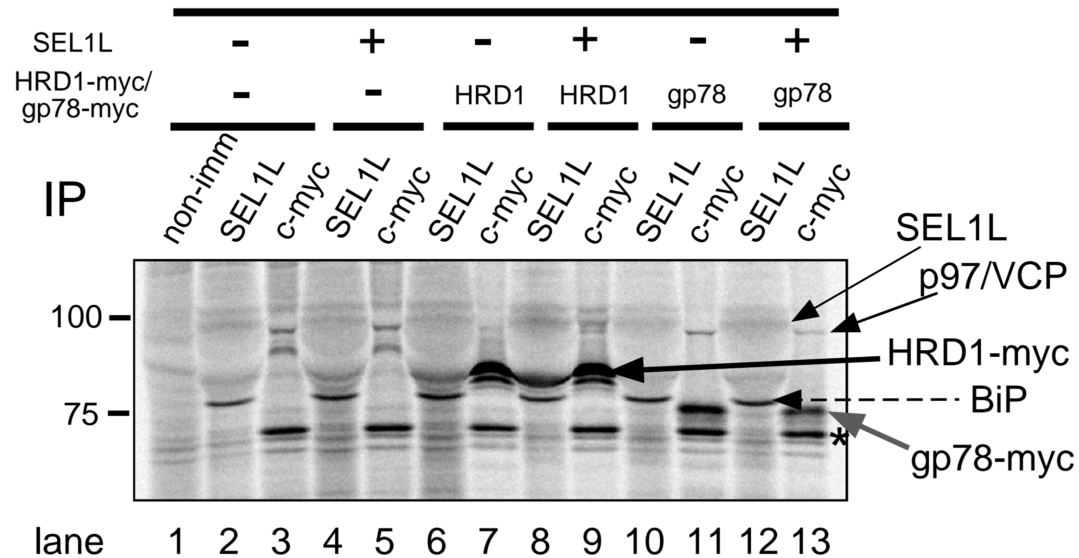
Suppl. Fig. 1. Co-immunoprecipitation of SEL1L and HRD1-myc. **(A)** Cells transfected with mock, SEL1L, and/or HRD1-myc/gp78-myc were labeled for 3 h with [^{35}S]-methionine/cysteine, extracted in buffer containing 3% digitonin, and immunoprecipitated as in Figure 2A. The asterisk indicates a non-specific band detected by Protein-G Sepharose beads. **(B)** As in (A), with the exception that the cells were extracted in buffer containing 1% NP-40 instead of 3% digitonin. Co-immunoprecipitation of SEL1L and HRD1-myc was readily detected in cells extracted with 3% digitonin (A, lanes 6-9), whereas the proteins did not co-precipitate when cells were solubilized with 1% NP-40 (B, lanes 6-9). The interaction between gp78 and p97/VCP was consistently detected in both lysis buffers (A, lanes 11 and 13; B, lanes 11 and 13). Co-immunoprecipitation of BiP was detected in cell lysates extracted with 3% digitonin (A, dotted arrow), but not those extracted with 1% NP-40. Over-expressed HRD1-myc was detected as a doublet, probably due to partial digestion of the N-terminus, since the C-terminal myc tag was detected in the faster migrating HRD1 band.

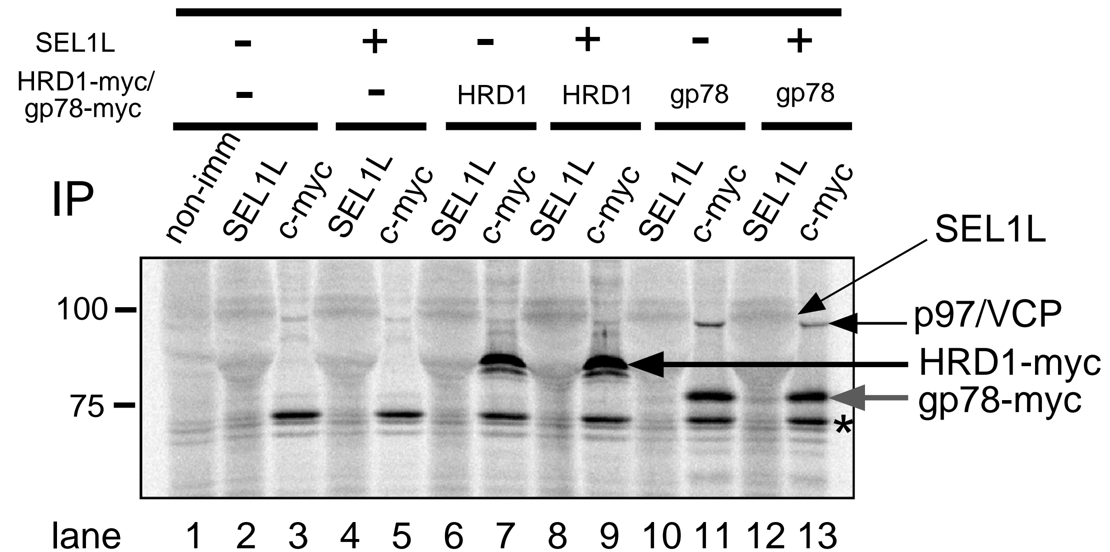
Suppl. Fig. 2. Knock-down of hXTP3-B and SEL1L by siRNA. **(A)** 293 cells plated on 35 mm dishes were transfected with 10 nM of the indicated siRNA. Following a 24 h incubation, cells were transfected with 0.5 μg of the hXTP3-B-short-FLAG and of the -long-FLAG plasmid. After an additional incubation for 24 h, the cells were harvested and aliquots of the cell lysate (1/10 volume) were subjected to Western blot analysis. The membrane was blotted with anti-calnexin (CNX) as a loading control. Since the XTP3B-1 siRNA was targeted to the sequence that is spliced-out in hXTP3-B-short mRNA, only hXTP3-B-long was knocked-down (lane 3). RNA duplexes of XTP3B-2 and -3 knocked-down both the hXTP3-B-long and -short forms efficiently (lanes 5 and 6). XTP3B-1 inhibited cell proliferation even at a concentration of 10 nM. **(B)** 293 cells plated on 35 mm dishes were transfected with 30 nM of the indicated siRNA, and cells were harvested 24, 48, and 72 h after transfection. Aliquots of the cell lysate (1/10 and 1/40 volume) were subjected to Western blot analysis for SEL1L and β-actin, respectively. The SEL1L-1 siRNA did not knock-down endogenous SEL1L (compare lanes 1-3), but inhibited cell proliferation quite severely even at a concentration of 10 nM. The apparent decrease in SEL1L at 48 and 72 h after transfection (lanes 5 and 13) was due to the inhibition of cell growth. RNA duplexes of SEL1L-2 and -3 knocked-down endogenous SEL1L efficiently.

Suppl. Fig. 3. Co-immunoprecipitation of BiP with NHK, NHK-QQQ, and non-glycosylated NHK (tunicamycin-treated cells). Cells transfected with NHK or NHK-QQQ were incubated in the presence (+) or absence (-) of 5μg/ml tunicamycin for 4 h prior to metabolic labeling. Cells were labeled with [^{35}S]-methionine/cysteine for 15 min, and chased for the indicated times. Tunicamycin was present during the pulse and chase periods in the "+" samples. Half of the cell lysate was immunoprecipitated with anti-BiP **(A)**, and the other half was immunoprecipitated with anti-α1AT **(B)**. BiP synthesis was greatly induced by tunicamycin treatment (A, lanes 7-9). Co-immunoprecipitation of NHK-QQQ with BiP was clearly detected (A, lanes 4-6), whereas co-immunoprecipitation of NHK with BiP was almost undetectable (A, lanes 1-3), even though NHK expression was higher than NHK-QQQ expression (B, compare lanes 1-3 vs 4-6). The same result was obtained using cells extracted with ATP-depletion buffer (data not shown).

Suppl. Fig. 4. Protease digestion of hXTP3-B-short and -long. 293 cells plated on 100 mm dishes were transfected with 5 μg of each plasmid as indicated. The cells were labeled for 3 h with [^{35}S]-methionine/cysteine, and chased in normal growth medium for 3 h. Cells were harvested

in buffer containing 1% NP-40, and aliquots of the immunoprecipitates were digested with 0.1 mg/ml chymotrypsin on ice for the indicated times (Incubation). Before separation by 12.5% SDS-PAGE, PMSF (20 nM final concentration) was added to the fractions to inactivate chymotrypsin. Arrows indicate the positions of the full-length hXTP3B-short-HA and -long-HA, and the asterisk shows a non-specifically detected band. The proteolytic fragments generated from the -short and -long isoforms are similar, but some differences were detected (compare lanes 6-8 vs lanes 10-12). However, the total radioactivity of all of the fragments in a given lane was almost equal to the radioactivity of the full-length protein before protease digestion (lanes 5 and 9), suggesting that most of the hXTP3B-long and -short was productively folded.





Supplementary Figure 1

A

48 h after Tf

plasmid mock hXTP3B-long-FLAG + hXTP3B-short-FLAG

siRNA - *Control-1* *Control-2* *XTP3B-1* *XTP3B-2* *XTP3B-3*

Blot: FLAG (light exposure) 75 50 37

hXTP3B-long-FLAG

hXTP3B-short-FLAG

(heavy exposure) 75 50 37

Blot: CNX 100

lane 1 2 3 4 5 6

B

24 h 48 h 72 h

siRNA *Control-1* *Control-2* *SEL1L-1* *SEL1L-2* *SEL1L-3* *Control-1* *Control-2* *SEL1L-1* *SEL1L-2* *SEL1L-3* *Control-1* *Control-2* *SEL1L-1* *SEL1L-2* *SEL1L-3*

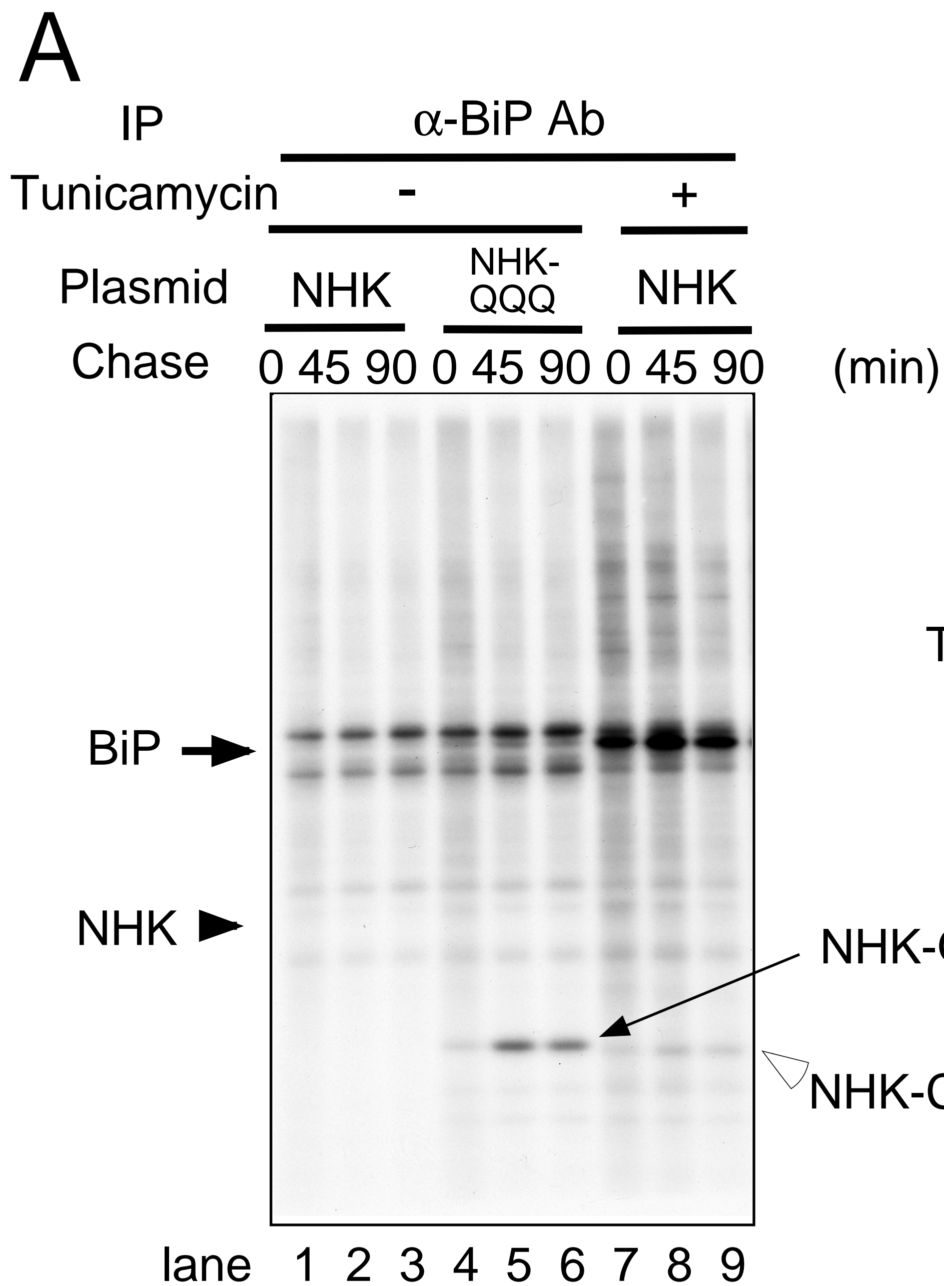
Blot: SEL1L 100 75

SEL1L

Blot: β-actin 50 37

lane 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

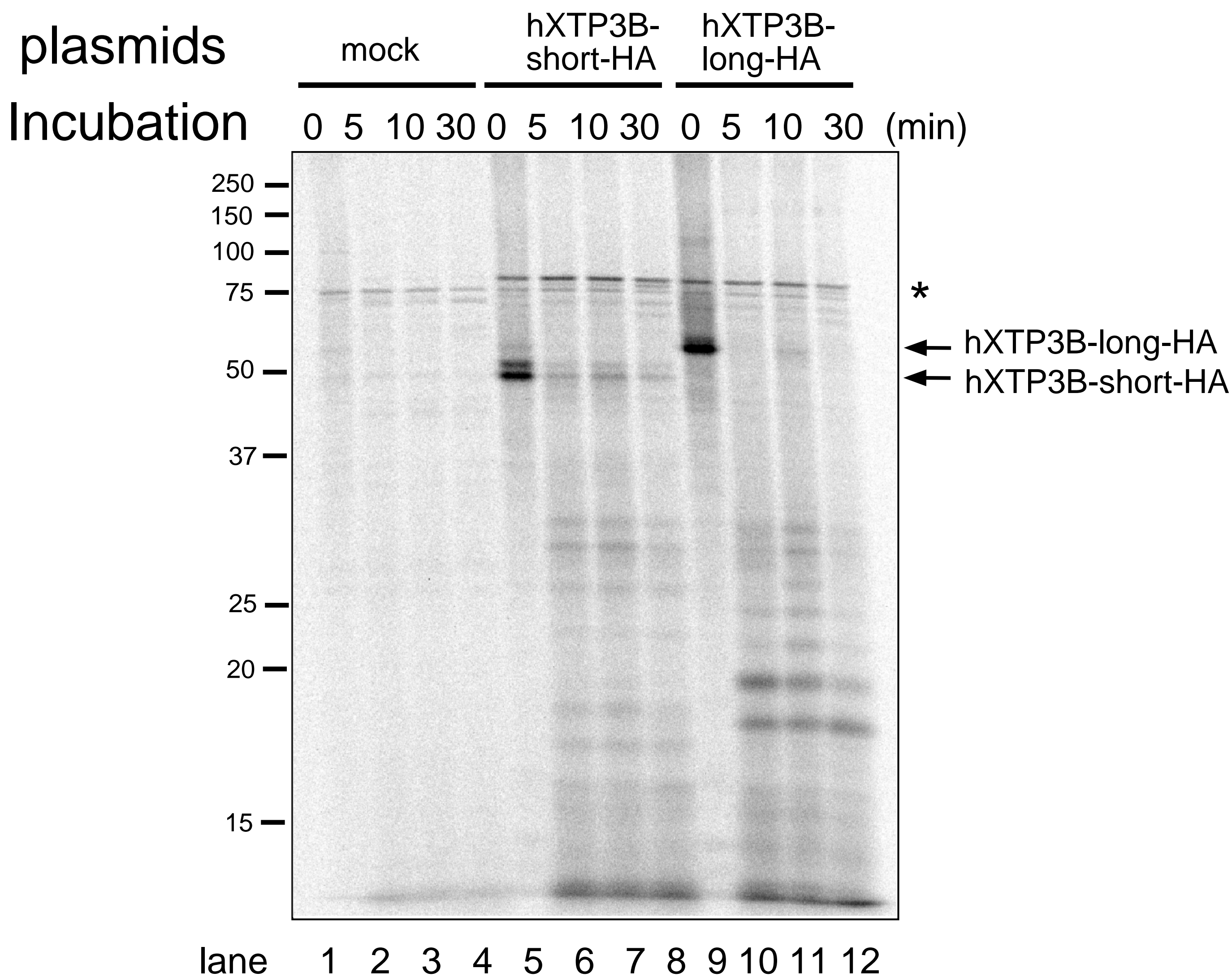
Supplementary Figure 2



B

IP
α-α1AT Ab
Tunicamycin
-
+
Plasmid
NHK
NHK-QQQ
NHK
Chase
0 45 90 0 45 90 0 45 90
(min)
NHK
NHK-QQQ
NHK-CHO
lane 1 2 3 4 5 6 7 8 9

Supplementary Figure 3



Supplementary Figure 4